"Prevalence of and Risk Factors for *Helicobacter pylori* Infection and Its Effect on Growth of Children in Mexico"

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By

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Facts, or what a man believes to be facts, are delightful . . . Get your facts first, and then you can distort them as much as you please.

Mark Twain

Science is built up with facts, as a house is with stones. But a collection of facts is no more a science than a heap of stones is a house.

Jules Henri Poincare

The great tragedy of science-the slaying of beautiful hypothesis by an ugly fact.

Thomas Huxley

Science is one thing, wisdom is another. Science is an edged tool, with which men play like children, and cut their own fingers.

Sir Arthur Eddington

Finding the occasional straw of truth awash in a great ocean of confusion and bamboozle requires intelligence, vigilance, dedication and courage. But if we do not practice these tough habits of thought, we can not hope to solve the truly serious problems that face us . . . and we risk becoming a nation of "suckers", up for grabs by the next charlatan that comes along.

Carl Sagan

ABSTRACT

Helicobacter pylori (*H. pylori*) infection causes achlorydria, depressed gastric acid barrier, impaired immune response and is suspected in bacterial overgrowth and diarrhoea. These features of the infection are known to cause significant malabsorption of nutrients and impairment of linear growth in children. The prevalence of *H. pylori* infection in children is known to be much higher in developing countries, especially among the lower socio-economic groups. The true prevalence of infection in urban children in Mexico and its impact on their growth are largely unknown. This study examined the prevalence of *H. pylori* infection in school children from an urban area in Northwest Mexico and attempted to identify the risk factors that predispose individuals to infection in childhood; as well as to relate the presence of this infection to growth of children. The cross-sectional study was conducted in 1997/98 in the poorest socio-economic sectors of the city of Hermosillo, Sonora, among 178 children aged 9 and 10 years. *H. pylori* status was determined in children by the ^{13}C-urea breath test. Anthropometric (weight and height) and haemoglobin measurements along with analysis of faecal samples and a 24-hour dietary recall were carried out in each child. Family socio-demographic/socio-economic status and living conditions data were elicited from parents by interview via structured questionnaires. The overall prevalence rate of *H. pylori* infection for the children in Hermosillo as determined by this study was 47.1%. The findings indicate that rural-born father, number of siblings, the type of main water supply (one tap in the yard) and the sharing of bed by the study child are important risk factors for acquiring the *H. pylori* infection. A borderline significant but small effect of *H. pylori* infection on height-for-age was observed in this study. *H. pylori* infection was found to be positively highly associated with *Hymenolepis nana*. No differences in mean energy, protein and iron intakes between *H. pylori* positive and negative children were observed. However, significant differences in the mean energy, protein and iron intakes were observed between boys and girls. *H. pylori* infection and enteric parasites were not significantly correlated with the presence of anaemia.

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INTRODUCTION

H. pylori is a gram-negative spiral-shaped bacterium that infects gastric-type epithelium, and is probably the most common bacterial agent in humans after *Streptococcus mutans*, which is implicated in dental caries. Evidence for *H. pylori* as a gastrointestinal pathogen is very strong. It is known that, the clinical spectrum of diseases caused by this bacterium ranges from asymptomatic carriers to such diseases as type B gastritis, and gastric and peptic ulceration.

The prevalence of *H. pylori* infection is higher in developing countries, especially in lower socio-economic groups. Infection of this bacterium in the gastric mucosa is known to occur in very young children (about 6 months age) with prevalence increasing as age advances. It is not known, however, if differences are the effect of various exposures and/or genetic susceptibility. Previous studies show lack of hot water tap and overcrowding in the childhood home as significant factors in adult seropositivity in developed countries and transmission by water and animals (in the home or farm). However, other studies report no increased risk of infection due to location of housing or source of water supply. The inconsistencies point out that the risk factors associated with *H. pylori* infection are not clearly understood.

Recent findings suggest an association between *H. pylori* infection in young children and diarrhoeal disease. An early sequela of *H. pylori* might be growth faltering. There are several mechanisms which need to be explored in order to understand the association between *H. pylori* and the role it plays in diarrhoea, malabsorption, and growth failure in children. An understanding of these issues is essential in order to design public health intervention strategies for dealing with this problem.

CHAPTER I

BACKGROUND

1.- What is *H. Pylori*?

In April 1982, an unidentified microaerophilic and gastric spiral bacterium was placed in the genus *Campylobacter* and named *Campylobacter pyloridis*. It was first cultured by Warren (1983) and Marshal (1983). Some time later, phylogenetic analyses based on 16S **rRNA** sequences and various other chemotaxonomic data provided incontrovertible evidence that the bacterium was not a member of the genus *Campylobacter* (Marshall and Goodwin, 1987; Goodwin et al, 1989) and it was then, designated *H. pylori*.

The latest phylogenetic tree shows that genus *Helicobacter* has fifteen (± 2) official species (Dewhirst et al, 1994). The species of interest, are the cluster of three gastric *Helicobacters*, shown to infect humans: *H. pylori*, *'H. heilmanii'* (previously called *'Gastrospirillum hominis'*), and *H. felis*. Although, *H. pylori* causes 99% of human *Helicobacter* infection, it is becoming clear that, the other two bacteria can also induce an active chronic gastritis even in asymptomatic individuals (Lavelle et al, 1994; Mazzucchelli et al, 1993).

H. pylori is a gram-negative, unipolar, multiflagellate, **S**-shaped bacterium, measuring 2-4 μm x 0.5-1.0 μm, but rod and ox forms can be observed *in vitro* (Marshall, 1983; Warren, 1983; Jones et al, 1984; Buck et al, 1986). It has a distinctive spiral-shaped appearance, and is recognised as a motile and non-invasive bacterium.

The organism has two to six polar flagella at the end of the cell, with a very characteristic and unusual morphology (Goodwin et al, 1985). Each flagellum consists of a central filament enveloped by a flagellar sheath. An observed characteristic of the flagella is the presence of a terminal bulb (Goodwin et al, 1985; Geis et al, 1989). Like many other spiral/helical bacteria, the morphology of *H. pylori* changes from helical form to spherical forms in extended cultures. Although, some researchers have proposed that these spherical forms may be a survival adaptation to adverse conditions in natural environments (Jones and Curry, 1990); their importance remains poorly understood.

As a microaerophillic bacterium, *H. pylori* generally inhabits the mucosa of the stomach. However it has been observed in adjacent oesophagus, and only one case in the rectum (Pambianco et al, 1988; Loeffield et al, 1992). After initial infection, the stomach may act as a reservoir from which the duodenum becomes colonised. The precise distribution of *H. pylori* in the stomach remains controversial, since it has been found in biopsies in both the antrum and the body, although it's preferred site appears to be the antrum (Dooley and Cohen, 1988; Axon, 1991). Located here, *H. pylori* appear to be of little consequence to the host, and most infected individuals remain asymptomatic in spite of the inflammatory change.

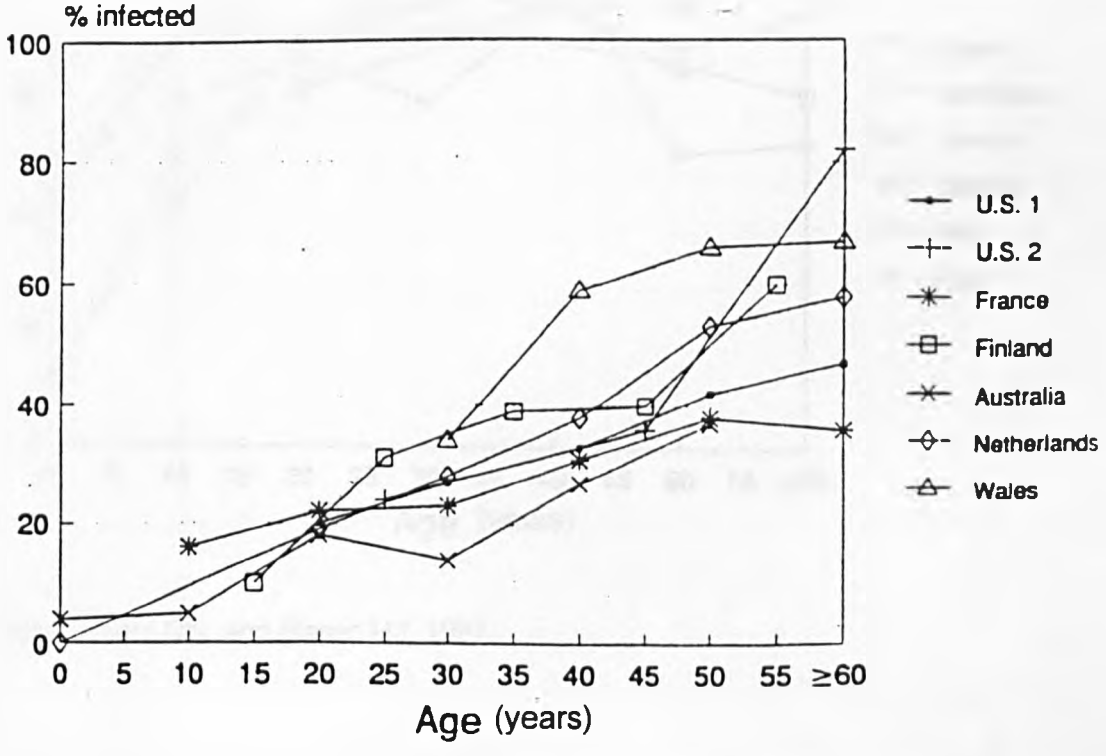
2. Challenging Epidemiological Findings of *H. pylori*

2.1. Prevalence

It is now clear that, *H. pylori* infection is present in all parts of the world.

The acquisition of infection, however, varies among and within populations (Figure 1 and 2). In developed countries (i.e. Europe, USA and Australia), it appears to be uncommon in childhood, whereas 50% to 60% of elderly adults may be infected (Jones et al, 1986; Graham et al, 1991; Matysiak-Budnik and Mégraud, 1994).

Figure 1. Relation between age and the prevalence of *H. pylori* infection among asymptomatic persons in developed countries

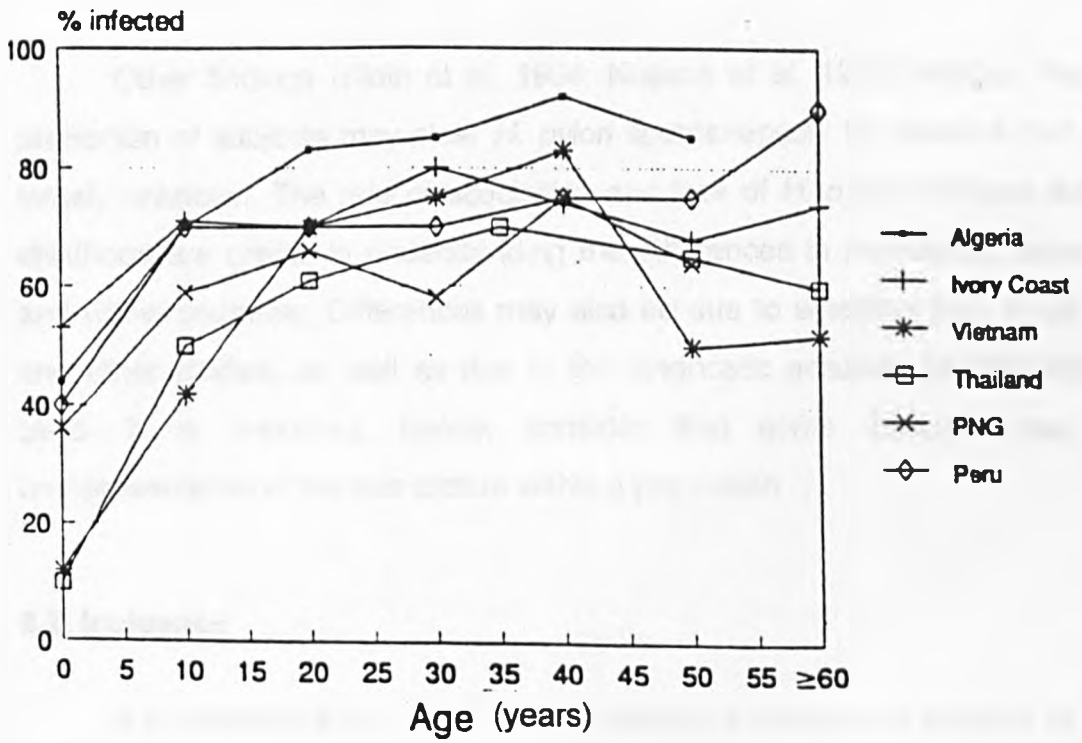


From: Taylor DN, and Blaser MJ, 1991.

Some reports suggest that, colonisation may occur during childhood, even in infancy in developing countries, and that prevalence continues to rise in adulthood (Wright et al, 1987; Miller et al, 1988; Katelaris et al, 1992; Mitchell et al, 1992; Weaver et al, 1995).

India (Katelaris et al, 1992) and North and West Africa (Wright et al, 1987; Miller et al, 1988) have the highest prevalence of *H. pylori* infection (97%, 70% and 82% respectively).

Figure 2. Relation between age and the prevalence of *H. pylori* infection among asymptomatic persons in developing countries



From: Taylor DN, and Blaser MJ, 1991.

In other regions such as South- Africa, Peru, Chile, Mexico and Thailand infection is acquired very early in life (Torres et al, 1998; Pelser et al, 1997; Ramírez-Mayans et al, 1997; Klein et al, 1994; Nurko et al, 1993; Hopkins et al, 1993; Klein et al, 1991; Klein et al, 1990; Perez-Perez et al, 1990). Whether, most of the infection occurs in childhood or is largely due to a cohort or generation effect remains poorly understood.

There are a number of questions concerning the acquisition of *H. pylori* during childhood. For example, in China (Mitchell et al, 1992), Viet Nam, Ivory Coast, Algeria (Mégraud et al, 1989) and Peru (Klein et al, 1991) overall *H. pylori* seropositivity rates are higher, and seroconversion occurs earlier than in the United States, England and France (Jones et al, 1986; Mégraud et al, 1989; Graham et al, 1991).

Other findings (Klein et al, 1994; Kuipers et al, 1993) indicate that, a proportion of subjects may clear *H. pylori* spontaneously for reasons that are largely unknown. The rate of acquisition and loss of *H. pylori* infection during childhood are critical in understanding the differences in prevalence between and within countries. Differences may also be due to selection bias in clinical and other studies, as well as due to the diagnostic accuracy of the method used. It is important, hence, consider that some findings may be unrepresentative of the true picture within a population.

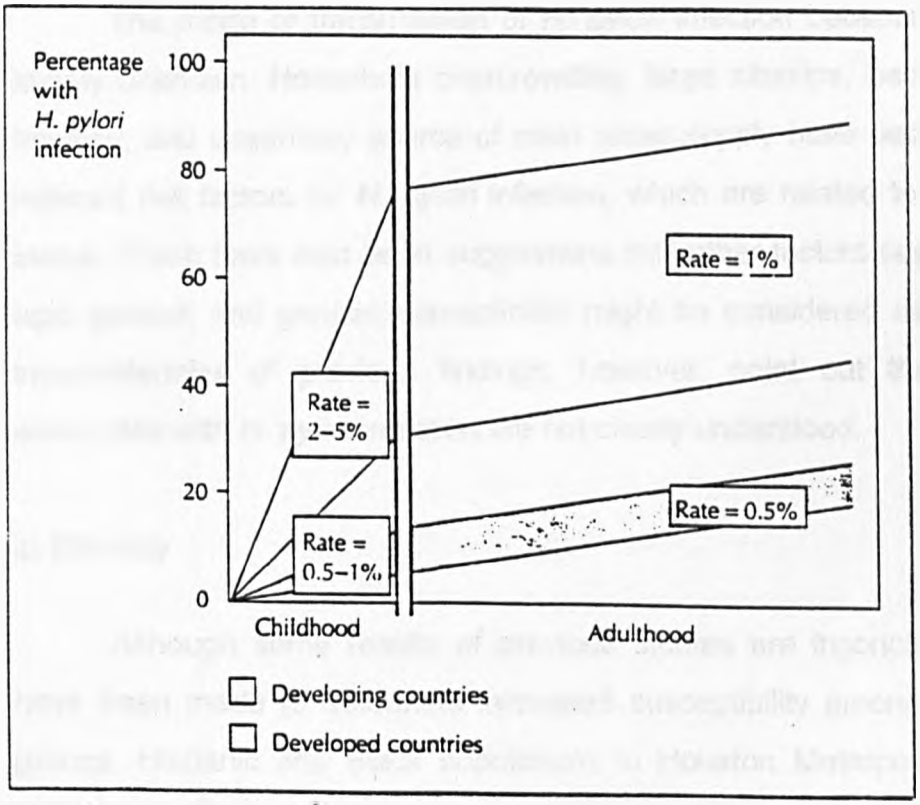
2.2. Incidence

It is assumed that once *H. pylori* infection is acquired, it persists for life. Although data on the incidence of *H. pylori* are limited, the latest findings confirm that rates are low during adulthood. Studies also reveal that, the rate of acquisition of this disease particularly in childhood varies widely in developed and developing countries, (Figure 3).

Adults in developed countries, have an annual incidence of infection of about 1 to 2 percent. One study in the United States, found an annual seroconversion rate of 0.49 percent (Parsonnet et al, 1992). In a cohort of Japanese, new cases appeared at a rate of 1.1% per year.

Among the youngest subjects however, the rate was two to three times higher (Banatvala et al, 1994). Another study in the Houston Metropolitan area, reported a rate of 1% (Graham et al, 1991; Malaty et al, 1992).

Figure 3. Trends in the rate of acquisition of *H. pylori* infection in developed and developing countries



From: Malaty et al, 1994. In: Axon ATR. *H. pylori* its role in gastro-intestinal disease. 1994.

For populations of developing countries, the incidence rate is unclear. Only studies conducted recently in two regions of China (urban and rural), report an incidence rate on the order of 1% (Mitchell et al, 1992), similar to that of adult populations in developed countries. Another study in Venezuela (Buiatti et al, 1994), however, observed a much higher re-infection rate.

The incidence of *H. pylori* infection appears to be higher in children than adults. This difference could be in theory possibly due to the fact that, adults currently infected are more likely to have acquired the infection in childhood.

2.3. Risk factors for *H. pylori* infection

The mode of transmission of *H. pylori* infection between individuals is largely unknown. Household overcrowding, large sibships, bed sharing, poor housing, and unsanitary source of main water supply have been identified as relevant risk factors for *H. pylori* infection; which are related to low economic status. There have also been suggestions that other factors such as ethnicity, age, gender, and genetic susceptibility might be considered risk factors. The inconsistencies of previous findings, however, point out that risk factors associated with *H. pylori* infection are not clearly understood.

a) Ethnicity

Although some results of previous studies are inconclusive, attempts have been made to determine increased susceptibility among certain ethnic groups. Hispanic and Black populations in Houston Metropolitan area were found to have seropositivity rates twice as high as the White population (Graham et al, 1991). A similar study in Los Angeles (Dehesa et al, 1991) found that, the frequency of *H. pylori* was higher in Blacks than in Whites. In both studies, the difference remained significant after adjustment for confounding variables such as age, sex, diet, educational level, income, and use of tobacco and alcohol.

In New Zealand (Morris et al, 1986), seropositivity among adults varied among persons of Tongan (70%), Samoan (44%), Cook Island (39%) and Caucasian (15%) extraction. Dwyer et al. (1988) found differences in antibody response in adult refugees on entry into Australia: 43% for Ethiopians, 40% for Salvadoreans and 18% for Vietnamese. Seropositivity rates (15%) among native-born Caucasians were found significantly lower by comparison. However, a population of Australian aborigines (where duodenal ulcers are rarely diagnosed) were found to have rates of only 0.5% (Dwyer et al, 1988). Similarly, other studies have also found differences in seropositivity in both New Zealand adult and children of Maori and Pacific Island ethnic groups, which persisted after adjustment for age and socio-economic status (Fraser et al, 1996).

Asian populations have also been found to have high seropositivity rates. Kang et al. (1990) report ethnic differences in Singapore; the Chinese have a rate seven times higher for peptic ulcer disease and twofold in antral gastritis and *H. pylori* infection compared with Malaysians. Similarly in China (Mitchell et al, 1992), in the Gansu Province (an area of high gastric cancer mortality) there was considerably higher overall prevalence rate of *H. pylori* infection than in the Guangdong Province (an area with low gastric cancer mortality). One study conducted recently in Malaysia (Goh KL, 1997) also found that the overall prevalence of *H. pylori* infection was higher in Chinese and Indians.

These differences among ethnic groups point out that factors such as cultural practices, environmental factors and/or an inherent genetic susceptibility may influence the rate of acquisition of infection independent of other factors such as socio-economic status.

Nevertheless, the results of prevalence could be over or under-estimated due to the sensitivity and specificity of the diagnostic test used in each study.

b) Socio-economic status

Socio-economic status as a risk factor has also been widely investigated. *H. pylori* prevalence (during childhood) is inversely associated with social class, which persists after controlling for numerous variables. However, results differ according to the standards used to assess socio-economic status such as family income, occupation, educational level and/or living conditions.

In a large random population of Welsh men (Sitas et al, 1991) the age standardised seroprevalence rates were lowest (49.2%) in combined social class categories I and II (based on occupation) compared to the lower social class, and with large differences within the youngest age group (30-34 years). It is impossible, however, to predict that young men in the higher social classes, will remain free of infection throughout life. Similarly, another study in Arkansas, in children found *H. pylori* infection to be more common in lower-income families than in high-income families (Fiedoreck et al, 1991).

Similar trends have also been observed in populations of developing countries. A higher *H. pylori* seroprevalence, was reported among Peruvian persons under 30 years of age attending public clinics in comparison to private clinics (Ramirez-Ramos et al, 1990). In a Chilean population the increase in overall prevalence rate of *H. pylori* infection began at 4 years of age in the lower socio-economic groups (Hopkins et al, 1993). In Saudi Arabia, a higher rate of *H. pylori* infections were reported in non-college graduates than in college graduates (Al-Moagel et al, 1990).

A high prevalence rate has also been reported among adults who were in a low socio-economic group during childhood, regardless of current social class. This information is consistent with the hypothesis that, once *H. pylori* infection is acquired it seems to persist for life. It is plausible, therefore, that differences observed in the general epidemiology of the infection among populations and generations could be explained by the relationship between childhood and present socio-economic status. Additionally, the inverse correlation between socio-economic class and high prevalence rates may be solely a reflection of poorer living conditions, rather than present family income.

c) Housing and living conditions

H. pylori infection rates have also been shown to be related to density of individuals in a household during childhood (Mitchell et al, 1992; Malaty and Graham, 1994; Webb et al, 1994). Two retrospective studies from developed countries have found a strong association between adult seropositivity and overcrowding in the home independent of father's social class (Mendall et al, 1992; Whitaker et al, 1993). These findings are consistent with data reported by one of the largest cross-sectional epidemiologic studies carried out in China (Mitchell et al, 1992).

The effect of sharing a bed in childhood has also been reported as a possible independent risk factor, regardless of age (Whitaker et al, 1993; Webb et al, 1994). Mendall et al (1992), have reported an association between lack of a hot water tap in the childhood home and seropositivity in adult life. Another study in Peru by Klein et al (1991), proposes drinking water as an important source of infection.

On the contrary, other studies have not found an increased risk of being infected due to factors such as type of housing, location of housing, source of water supply, hot water supply, or an indoor toilet (Fiedorek et al, 1991; Mitchell et al, 1992; Whitaker et al, 1993). These inconsistencies point out that the socio-economic status, family size and overcrowding in the childhood home may only be markers for some other environmental factors or cultural practices, which indirectly affect the mode of transmission of *H. pylori* infection.

d) Genetic

An alternative explanation for enhanced susceptibility to *H. pylori* infection may be genetic factors. Only one cross-sectional study on twins (Malaty et al, 1994) has suggested that genetic factors influence the acquisition of *H. pylori* infection, but sharing the same rearing environment is a significant factor. Other studies (Rotter et al, 1979; Mossi et al, 1993) have attempted to demonstrate a genetic basis for *H. pylori*-related disorders, such as gastritis, peptic ulcer disease and gastric cancer. Such evidence, however, is not strongly as reported by Malaty et al, (1994). One obstacle to establishing the possible inherent genetic susceptibility to increased risk of *H. pylori* infection is the inability to eliminate entirely environmental influences.

e) Gender

Another risk factor could be biologic sex. Although infection of *H. pylori*, seems to be equally common among men and women, studies report differences. One study in California (Replogle et al, 1994), reports *H. pylori* infection to be more prevalent in men than in women (aged 20 to 39 years); the difference remained after controlling for ethnicity, income and education.

Similarly, a study in three different geographic regions of Peru found that, the frequency of infection in women appears to be approximately 10% less than that of men (The Gastrointestinal Physiology Working Group of the Cayetano Heredia and The Johns Hopkins University, 1992). Other investigations conducted in Colombia have also found a greater prevalence in boys compared with girls (2.0-9.5 years old), which persisted after adjustment for parent's occupation (Goodman et al, 1996). A large epidemiological study in two regions of China (Mitchell et al, 1992), and another in children in Arkansas (Fiedoreck et al, 1991) reported no significant difference between males and females. The possible effects of gender may be explained by differences of age in the study populations. Hence, gender, as an important risk factor will have to be examined in greater detail in the future.

f) Diet

Diet and specific dietary components are among environmental factors most likely to have a possible protective and/or adverse role on gastrointestinal mucosal integrity. Two studies have shown some potentially beneficial effects of dietary lipid on the stomach but as a consequence of mucosal prostaglandins (Tarnawski et al, 1987) and on mucosal (Dial and Lichtenberger, 1987). In contrast, caffeine has been associated with enhancement of gastric acidity (Cohen et al, 1971). Additionally, excess salt intake has been found to cause mucosal damage (Correa, 1992). The data, which remain uncorroborated and conflicting, point to the limited amount of information on dietary components associated with *H. pylori* infection.

It is well known that, *H. pylori* is able to induce an inflammatory response in human gastric antral mucosa, presenting chemotactic activity for neutrophils and increasing the production of reactive oxygen species.

Thus, nutrients for antioxidant defense such as ß-carotene, vitamin E and vitamin C, may play a relevant role preventing oxidative damage to the gastric mucosa by quenching reactive oxy-radicals.

Sobala et al (1989), report lower gastric levels of total vitamin C and ascorbic acid in subjects with type B chronic gastritis and evidence of *H. pylori*, compared with subjects having a normal gastric mucosa. In addition, there was a vitamin C concentration gradient from gastric juice down to plasma in subjects with normal gastric mucosa, but not in those with chronic gastritis. Two years latter Sobala et al (1991), reported a case of acute infection with *H. pylori* accompanied with a sustained fall in gastric juice ascorbic acid concentration. Whether lower gastric vitamin C levels, in the presence of chronic gastritis by *H. pylori*, are due to enhanced utilisation to prevent oxidative damage in gastric mucosa; diminished secretion; or back-diffusion through damaged mucosa, is still unknown.

Recently an *in vitro* dose-reponse study, with two of the most common dietary polyunsaturated fatty acids (linolenic, and arachidonic) showed direct effects on inhibition of growth of *H. pylori* and its motility (Thompson et al, 1994). Whether these effects are present in the natural habitat of the bacterium, will have to be corroborated using either *in vivo* or epidemiological studies.

2.4. Modes of transmission of *H. pylori* infection

a) Animal-to-man spread

To date, humans seem to be the sole natural host of *H. pylori*. However, hosts other than humans could harbour *H. pylori*.

Some attempts have been made to identify animal reservoirs of the bacterium (Goodwin et al, 1989; Otto et al, 1994; Handt et al, 1994).

Gastritis naturally occurring and associated with *H. pylori* has been observed in some species of monkey such as Macaca mulata and M. nemestrina (Bronsdon et al, 1988), in baboons (Baskerville and Newel, 1988) and in swine (Rivera et al, 1989). These findings, however, do not explain the widespread infection of *H. pylori* since human contact is infrequent with many of these animals.

Recently Handt et al (1994), found *H. pylori* in a colony of cats. Transmission of the bacterium between cats and humans may play a role in the epidemiology of infection. Similarly, a previous study reported that, *H. pylori* colonised the gastric mucosa of germ-free dogs and was transmitted by contact from infected to uninfected dogs (Radin et al, 1990). Such evidence raises the possibility that, *H. pylori* infection may be a zoonotic disease. Whether animals might be a reservoir for human infection, remains to be elucidated in future studies.

Indirect evidence from a epidemiologic study, has also shown higher seropositivity rates in Italian abattoir workers than in subjects who had no exposure to animal parts (Vaira et al, 1988). There were many confounding variables, however, making it difficult to draw definitive conclusions.

b) Person-to-person spread

The lack of an environmental reservoir for *H. pylori* and epidemiological evidence suggest that person-to-person transmission of infection occurs during childhood.

Findings of intra-familial clustering of *H. pylori* infection (Mitchell et al, 1992; Malaty and Graham, 1994; Webb et al, 1994) as well as among residents of closed communities (Berkowicz and Lee, 1987; Reiff et al, 1989; Perez-Perez et al, 1990), provide evidence in support this hypothesis. Some factors previously reported, such as crowded living conditions, and sharing a bed in childhood and closed environments may play an important role in transmission by person to person contact.

Apparently, the transmission of each strain from person to person generally occurs within families but primarily in childhood. Children infected with *H. pylori* always come from a family that contains at least one seropositive adult (Malaty and Graham, 1994). Drumm et al (1990) found that parents, mainly mothers, might be a source of infection. Conversely, Klein et al (1989), did not find correlation between *H. pylori* status of parents and child. A large study in New York among 277 couples visiting an infertility clinic, found no increased rate of infection among the spouses of seropositive index cases (Perez-Perez et al, 1991). Similar findings were reported by Mendall et al (1993), and Jones et al (1987). The data on the person to person spread seem rather inconsistent.

In addition, some studies on the strains isolated from various family members by DNA and RNA typing have reported different results. Nwokolo et al (1992), report the occurrence of clonal variants of the same strain in three generations of family members. Bamford et al (1993), studied seven members of three families and found identical strains present in parents and the son of one family but not in the other two families. Simor et al (1990), found different strains in each of two pairs of siblings. Finally, Tee et al (1992), found variability in strains detected among members of five families of seven families studied. The findings of different strains of *H. pylori* within a family may only mean the possible genetic evolution of the bacterium.

The data on the person-to-person transmission of *H. pylori* does not provide the strongest evidence but confirms the spread of infection within families. Nevertheless, they have failed to find a common exogenous source for different family members. Hence, controlled studies of differences in living conditions might allow identification of factors that directly affect transmission, including factors outside the home.

Indirect person-to-person transmission predominantly via endoscopes, has been recently reported (Langenberg et al, 1990). Thus, iatrogenic acquisition of *H. pylori* infection might be a common mode of transmission. It is consistent with previous findings that endoscopists not using gloves have an increased rate of infection (Mitchell et al, 1989).

c) Oral-oral spread

Although a clear route has not yet been identified, the epidemiological evidence suggests that oral-oral and faecal-oral routes can occur, in spite of *H. pylori* not being isolated from the physical environment.

The hypothesis of oral-oral spread is based on results in experimental and epidemiological studies. As mentioned previously, *H. pylori* was transmitted by contact from infected to uninfected dogs, suggesting a possible oral-oral transmission (Radin et al, 1990). A study in West Africa reports increased rates of infection in children due to maternal pre-mastication of food (Albenque et al, 1990). Additionally, in Australia the highest seroprevalence for *H. pylori* was found among Chinese immigrant people who used chopsticks for eating, which is associated with shared food dishes within families (Chow et al, 1995).

Oral-oral transmission is plausible since *H. pylori* has also been cultured from dental plaque and saliva in both healthy and symptomatic subjects (Krajden et al, 1989; Shames et al, 1989; Banatvala et al, 1993; Pytko et al, 1996). An increased rate of infection, however, has not been reported among dental workers (Malaty et al, 1992; Banatvala et al, 1995).

d) Faecal-oral spread

H. pylori has been detected in faeces and it is claimed that a normal method of infection is via the faecal-oral route (Thomas et al, 1992; Luzzi et al, 1993). This is based on an inference analogous to hepatitis A known also to be transmitted by this route (de Korwin et al, 1982; Lee et al, 1991). A study in homosexual and heterosexual men in Denver, however, did not find significant differences in the lifetime number of sex partners or in history of previous sexually transmitted diseases among infected and uninfected individuals. This suggests that the sexual transmission of *H. pylori* infection is unlikely (Polish et al, 1991).

A study in Chile suggested that irrigation water contaminated with *H. pylori* from sewage could contaminate vegetables and so serve as one faecal-oral route of transmission (Hopkins et al, 1993). Whether favourable conditions can enhance survival time of *H. pylori* in the external environment remains unknown. However, *in vitro* experiments have demonstrated that, *H. pylori* survives for several days in sea water, saline and distilled water (West et al, 1990). Another study found that the bacterium was capable of growing under aerobic conditions with high humidity (Xia et al, 1994). Recently, another study reported that *H. pylori* is quite sensitive to desiccation and is not likely to survive well in a dry environment (Jiang X and Doyle MP, et al 1998).

This information may have significant implications in epidemiological research regarding routes of transmission.

e) Foodborne transmission

It is possible for *H. pylori* infection to be transmissible through food-associated vehicles. Only one study has attempted to assess the viability of *H. pylori* in drinking water and the findings were that the bacterium's cocoid form could remain for up to 30 days (Sahamat et al, 1993). Klein et al, (1991) reported in Peruvian children that drinking water could be an important source of infection and vehicle for transmission. Although, these two findings give an indication that *H. pylori* may persist in water in a viable form, further studies are necessary to confirm or refute a waterborne route as an important means in the transmission of this disease.

Additionally, one study in Chile reported that *H. pylori* seropositivity was associated with consumption of raw vegetables and associated with contamination by irrigation water polluted with sewage (Hopkins et al, 1993). Recently another *in vitro* dose-reponse study reported that *H. pylori* may be neither robust nor capable of growing in many types of foods because it cannot grow at 25 ° C in 2% NaCl or at a water activity of 0.96 (Jiang and Doyle, 1998). These conflicting evidences are not compatible with food-borne transmission. Well-designed epidemiologic studies are needed before food-borne transmission can be ruled out.

The inconsistency of the findings discussed above shows that the mode of transmission, the source of infection, as well as risk factors both in the individual and within households are largely unknown. Hence, these *H. pylori*-related issues remain a challenge for future research.

3.- The Clinical Spectrum of *H. pylori* Infection in Childhood

3.1. Asymptomatic infection

Although *H. pylori* causes an inflammatory response, most infected persons do not have symptoms. As in adults (Barthel et al, 1988; Peterson et al, 1988), *H. pylori* infected children are often asymptomatic. Thomas and colleagues found *H. pylori* antibodies in 5% of healthy schoolchildren, and 4% of children in hospital with non-gastroenterological symptoms (Thomas et al, 1992). In Arkansas, Fiedoreck et al (1991), found that 24% of asymptomatic children at ages 3 to 5 years were colonised, which increased to 45% at ages 16 to 20 years. Most recently Blecker et al (1993) reported similar results in symptomatic children.

A large epidemiologic study in China reports *H. pylori* antibodies in 23% of healthy children at age 5, increasing with age (Mitchell et al, 1992). In spite of such findings, the true prevalence of infection in healthy children is uncertain because most studies assessed only a symptomatic population.

3.2. Non-ulcer dyspepsia and gastritis

The association of *H. pylori* with type B gastritis and peptic ulcer with or without dyspepsia is well documented in adults and children (Cadrenal et al, 1986; Czinn et al, 1986), although, it has never been clearly shown that upper gastrointestinal symptoms are more common in *H. pylori* infected populations than uninfected populations.

H. pylori infection has been implicated in 30-70% of adults with non-ulcer dyspepsia (vague upper abdominal discomfort, mainly nausea and vomiting) and active gastritis (Talley and Phillips, 1988; Blaser, 1990). However, the lack of universally accepted criteria for determining whether the subjects have non-ulcer dyspepsia or not, makes it difficult to ascertain a true rate of *H. pylori* associated with non-ulcer dyspepsia. In addition, non-ulcer dyspepsia is caused by more than one aetiology.

Several studies have attempted to provide information of symptoms in *H. pylori* infected children but most contain highly selected sample. Symptoms appear to diverge not much from those found in adults. Recurrent epigastric pain is the most reported feature in children.

Studies carried out by Drumm et al (1987), Mahony et al (1988), and Oderda et al (1989), report that children complaining of epigastric pain were infected with *H. pylori*. Mitchell et al (1983), reported abdominal pain in four infected children as major the symptom, but in two other children, epigastric burning, reflux, burping, periodic nausea, epigastric distension, flatulence, and halitosis were major symptoms. On the contrary, Glassman et al (1989), and Mahony et al (1992), found that, the presence of epigastric or abdominal pain and vomiting did not discriminate between children with *H. pylori* colonisation or gastritis, and those with normal gastric mucosa.

Other evidence supporting an association between *H. pylori* gastritis and recurrent abdominal pain in children are based on improvement in symptoms after therapy (Oderda et al, 1989; De Giacomo et al, 1990). An earlier study reported improvement in symptoms in children who had duodenal ulcer disease but not in those with gastritis alone (Drumm et al, 1988).

Evidence on whether *H. pylori* infection is related to the symptoms or whether the symptoms improve with clearance of bacterium, is unclear.

H. pylori associated symptoms could be easily misdiagnosed or missed, since chronic recurrent abdominal pain is a common complaint among children. Therefore, the true prevalence might be underestimated. In a more positive light, *H. pylori* associated symptomatology should be easier to identify in children than in adults because confounding variables such as smoking, alcohol, and drugs generally do not exist.

The association between peptic ulcers and *H. pylori* seems to be relatively rare in childhood. *H. pylori*-related lesions in the child differ from adults, in that endoscopic abnormalities are usually found in the antrum and show a micronodular aspect (Oderda et al, 1989; Chong et al, 1995). The clinical symptoms in children, however, do not differ from their presentation in adults. Bleeding, vomiting and most frequently epigastric pain are the chief symptoms reported in children (Czinn et al, 1986; Drumm et al, 1988; Yeung et al, 1990; Raymond et al, 1994).

At the same time, a family history of duodenal ulcer has been found both in children with duodenal ulcer and those with gastritis (Nwokolo et al, 1992). Whether this is due to a common exposure to a source of infection within families remains to be elucidated.

3.3. Nutrition-related disorders

H. pylori and its role in gastrointestinal diseases have raised much interest since its association with active chronic gastritis has been discovered.

There is little information, however, about the nutritional disorders caused by this infection (Table 1). Nevertheless, this bacterium may cause diarrhoea, malabsorption and growth failure in children.

a) Protein-losing enteropathy

Hill et al (1987) have reported an association between acute gastritis, *H. pylori* and severe protein-losing enteropathy. Three pre-school children from South Africa presented over a period of four months acute transient protein-losing enteropathy due to gastritis. Protein-losing enteropathy was confirmed by ^{51}Cr-labelled albumin studies. Resolution of the gastritis and the disappearance of H. pylori accompanied recovery from protein-losing enteropathy. Stool microscopy and culture did not show any parasites or bacterial pathogens; however, there was no virological examination. It is difficult, therefore, to establish a causal relationship between bacterium, gastritis and protein-losing enteropathy.

Cadranel et al (1991), observed a similar case (9-month-old) of protein-losing enteropathy due to acute gastritis but *H. pylori* was not identified. Both gastritis and protein-losing enteropathy healed spontaneously in 4 weeks. Although protein-losing enterophaty is caused by more than one aetiology, this does not rule out *H. pylori* infection. Protein loss via the intestinal wall seems to be one of the most accurate parameters to measure the inflammatory activity in the intestine.

Further studies of the relation between protein-loss and *H. pylori*-related acute gastritis in children is needed. There are a variety of non-invasive screening tests to measure intestinal permeability based on labelled macromolecules, which might be used to investigate this matter.

Table 1.- Association between *H. pylori* and its possible effect on nutritional status in childhood

Source	Study Design	Variables	Conclusions
Hill ID, et al., 1987 Three 2- to 4 1/2 year-old children (South Africa). Histology and culture of biopsy specimens.	Case Report	Gastritis and, protein losing enteropathy	Association between gastritis caused by *H. pylori*, and protein losing enteropathy
Sullivan PB, et al., 1990 Twenty 1 1/2- to 2 year-old children (The Gambia). Serology and histology.	Cross-sectional survey (age matched group)	Chronic diarrhoea and malnutrition, weight and, height	Chronic diarrhoea and malnutrition were associated with high anti-*H.pylori* antibody titres
Nurko SS, et al., 1993 One hundred and twenty four 4/12- to 3 year-old children (Mexico). Serology.	Prospective Follow-up period not reported	Persistent diarrhoea and, malnutrition	*H. pylori* appears to be a significant risk factor for persistent diarrhoea independent of malnutrition
Raymond J, et al., 1994 Four hundred and twenty six 2 days- to 16 year-old children (France). Histology and culture of biopsy specimens.	A 2-year follow-up (age matched group)	Clinical symptoms of *H. pylori* infection, weight loss and, short stature	Predominance of infection among male rather than female children, and *H. pylori* present in 55.2% children being examined because of short stature
Patel P, et al., 1994 Five hundred and fifty 7 year and 11 year-old children (Edinburgh). Serological test (saliva).	Follow-up (two years)	Height, indicators of socio-economic status and, family structure	Growth in height between 7 and 11 year was diminished in infected children (mean 1.1 cm) and was largely confined to girls

Table 1.- Association between *H. pylori* and its possible effect on nutritional status in childhood

Source	Study Design	Variables	Conclusions
Weaver LT, 1995 Two hundred and forty eight 3 to 45 months-old children. (The Gambia). ^{13}C-urea breath test.	Follow-up (at three monthly intervals for two years)	Diarrhoea and, malnutrition	Malnutrition did not predispose to infection, but rather the converse (infection was followed by diarrhoea and growth faltering)
Oliveira AMR, et al, 1994 Two hundred and forty nine 1month to 18 year-old children. (Brazil) Serology	Cross-sectional survey	Weight and height, indicators of socio-economic status and, family structure	There was no significant difference in the prevalence of *H. pylori* infection related to nutritional status
Mahalanabis D, et al, 1996 Four hundred sixty nine 1 to 99 months-old children. (Bangladesh) ^{13}C-urea breath test	Cross-sectional survey	Weight and indicators of socio-economic status	Overall *H. pylori* infection had no association with nutritional status of child
Clemens J, et al, 1996 Five hundred and sixty nine 2 to 9 year-old children. (Bangladesh) Serology	Cross-sectional survey	Weight and height and family structure	Infected children did not differ significantly from non-infected children in Z scores for weight-for-age or height-for-age
Perri F, et al, 1997 Two hundred and sixteen 3 to 14 year-old children. (Italy) ^{13}C-urea breath test.	Cross-sectional survey	Weight and height, indicators of socio-economic status and, family structure	Centile value for height was significantly related to *H. pylori* status in children aged 8.5 to 14 years

b) Diarrhoeal disease

It appears that there is an association between *H. pylori* infection and persistent diarrhoea in children as reported in two studies. Sullivan et al (1990), report *H. pylori* infection in Gambian children aged under 5 years, using serologic tests. They found a significant increase in the prevalence rate of *H. pylori* infection in children with chronic diarrhoea and marasmus (53%) in comparison with matched control groups (26%). However, *H. pylori* were only demonstrated histologically in 10 of the children with chronic diarrhoea and malnutrition out of 17 who underwent endoscopy. Similar results are reported by Nurko et al (1993), in children 4-35 months old from Mexico. They determined antibody levels by using a commercial IgG ELISA test for *H. pylori*, and found it more common in children with persistent diarrhoea (40%) than in those with malnutrition but without persistent diarrhoea.

Both Sullivan et al, and Nurko et al, lacked detailed parasitic, bacterial and, virological examination in stools, making it difficult to establish a direct link to *H. pylori* in the pathogenesis of diarrhoea. These findings are confounded by the fact that selected children were malnourished who could have developed diarrhoea following another pathogenic agent. *H. pylori* physiopathology, however, may be a predisposition for developing diarrhoeal disease. More studies are needed to explore these associations.

Human ingestion studies in adults have shown that acute *H. pylori* infection results in transient hypochlorhydria (Morris and Nicholson, 1987). Diminished gastric acid that persists for more than a year has been reported in several cases (Gledhill et al, 1985).

One can assume that a considerably raised fasting gastrin value observed in young children with *H. pylori* gastritis may reflect achlorhydria associated with acute infection (McCallion et al, 1995). Thus, it may be a predisposing factor to recurrent diarrhoeal disease by small bowel bacterial overgrowth.

c) Growth failure

An early sequela of *H. pylori* infection might be growth faltering. Several studies have commented on the finding that *H. pylori* infected children are shorter (stunting) and have lower weights (wasting) than the uninfected. In two reports, from Peru (Klein et al, 1991) and the United Kingdom (Patel et al, 1994) the findings showed that the height to age ratio was significantly lower in children infected with *H. pylori* than in uninfected children, with the largest effect confined to girls in the United Kingdom. Raymond et al (1994), found that over half the French paediatric population being examined for short stature were infected with *H. pylori*. Although, they did not exhibit any sign of hypoproteinemia or malabsorption, they did have an abnormal gastric mucosa or clinical symptoms.

Most recently Weaver et al (1995), reported the nutritional status of a group of Gambian children at the time of seroconversion and compared it with age, and sex matched controls. They found that malnutrition did not predispose the study population to *H. pylori* infection, rather the reverse. *H. pylori* infection was the primary event, which was followed by diarrhoea and growth faltering. In addition, infants who developed *H. pylori* infection early in life showed significantly poorer weight gains during the first 18 months, in spite of a similar birth weight to those who did not acquire it after one year.

Based on these findings it is clear that there is not enough evidence to establish cause and effect between *H. pylori* infection and diminished growth in children. Nevertheless, an understanding of the interactions between infection and growth by a multiplicity of etiological factors is needed. Attempts must be made to provide new data. It is important to determine the true prevalence of *H. pylori* infection associated with malnutrition in developing countries. This will be difficult unless we are able to minimise suspected confounding variables such as dietary intake, intestinal parasites, nutritional iron deficiency, and quality of the family environment (psychological, psychosocial and emotional aspects).

Several trials conducted on undernourished children have shown that growth, physical fitness and appetite improved after therapy for parasitic infections such as *hookworm, trichuris trichura and ascaris lumbricoides* (Willet et al, 1979; Stephenson et al, 1993). It is unknown however, how much wasting and stunting on a global basis could be alleviated by efficacious chemotherapy and prophylaxis of *H. pylori* infection, or the extent to which it is responsible for child growth faltering. Therefore, efforts should address the effects of antibiotics on growth in *H. pylori* infected children.

4. Implications of *H. pylori* Infection on Nutritional Status of Children

The virulence factors of *H. pylori* allow for colonisation, morphological damage and disruption of gastric and intestinal cell function in the host (Table 2 and 3). Although *H. pylori* always induces an inflammation, a high percentage of infected subjects (especially children) remain asymptomatic. Hence, it is important to consider the pathogenic mechanisms of bacteria, which may cause disorders that have not been identified.

Table 2. Pathogenic factors of *H. pylori* and their toxic effects on surface epithelium

Pathogenic Factors	Virulence Mechanisms
Motility	To evade both gastric acidity and peristalsis
Adhesin (N-acethylneuraminyllactose-binding fibrillar hemagglutinin)	Responsible for binding to red cells
Enzymes:	
Urease	Destroys the lipoprotein layer and breaks hydrogen bonds of gastric mucosal barrier To neutralize gastric acidity in the microenvironment of the bacterium Ammonia produced may be a potent cellular toxin
Catalase	It could protect the bacterium from endogenous hydrogen peroxide produced by polymorphs
Phospholipases A_2 and C	May disrupt the normal phospholipid bilayer of the ephitelial cell membrane and affect cellular integrity
Proteases	Degrades mucin in vitro
Alcohol dehydrogenase	Acetaldehyde production may causes gastric mucosal damage
Arachidonic acid	Is converted into leucotrienes and other eicosanoids, increasing membrane permeability
Vacuolating cytotoxin with an Associated gene (cgA)	Associated with the severity of infection and can induce the secretion of cytokine interleukin-8 (recruitment of neutrophils)
Human lactoferrin receptor system	Iron acquisition from the host

Table 3. Interrelated anatomical and physiological changes in the gastric lumen and mucosa caused by *H. pylori*

	Changes	Effects
Gastric Lumen	Hypochlorhydria	↑ N-nitroso compounds (nitrosamines and nitrosamides)
	↓ Vitamin C	↑ N-nitroso compounds
	↑ Acetaldehyde (oxid. of alcohol)	↑ Gastric mucosa damage
	↑ Ammonia (urease activity)	Inhibits cellular respiration, causes acute cytotoxic effects and accelerates epithelial proliferation
Mucosa and submucosa	↑ Cell turnover	↑ Lymphocytic infiltration and mutagenesis
	↑ Reactive oxygen metabolites from neutrophils	↑ Genotoxicity and mutagenesis
	↑ Cytokines:	
	Interleukin-1	↑ Mucosal inflammation by the stimulation of arachidonic acid metabolism and excessive gastrin release
	Interleukin-6	Excessive gastrin release and pyrogen
	Interleukin-8 TNF-α	↑ PMN neutrophil chemotaxis, leading to tissue damage
	Interferon	↑ Mucosal inflammation
	Platelet-act.fact.	Leading to occlusion of the Microcirculation and loss of ephitelial Integrity by ischaemic damage

H. pylori-related luminal and mucosal factors, and immunophysiologic changes may play a significant role in the development of diarrhoea, malabsorption, and general nutritional deterioration of infected children. The true role of these mechanisms of infection waits to be investigated.

4.1. Mechanisms of diarrhoea and malabsorption of nutrients

Mucosal integrity is of prime importance in maintaining a healthy state in humans because it performs as a deterrent to microbial and/or toxin attachment to mucosal surface (mucus coat and microvillous membrane). *H. pylori* results in inflammation by invasion, and destroys mucosal cells with its cytotoxic products, leading to gastrointestinal pathology. It is well recognised that most gastrointestinal infectious agents cause diarrhoea by four general mechanisms: enterotoxin production; cytotoxin production, injury of the microvillous surface via mucosal adherence (not through invasion of the mucosa); and intestinal mucosa invasion. Thus, *H. pylori* infection itself may predispose the individual to diarrhoeal states by compromising the normal morphology and physiology of the stomach and upper intestine, or bacteria might be causing a symbiotic state by which the host is unaffected.

Although the relationship between hypochlorhydria and gastritis associated with *H. pylori* is controversial, acid secretion decreases after first infection. Decreased gastric acid secretion contributes to small bowel contamination (Giannella et al, 1973 and 1977; Gilman et al, 1988) resulting in bacterially induced diarrhoeal states. *H. pylori*-related hypochlorydria may lead to gastric/small bowel bacterial overgrowth, thereby predisposing the individual to enteritis. This could represent an important pathogenic condition (chronic diarrhoea) already observed by Hill et al (1987), Nurko et al (1993), and Weaver (1995).

Having colonised the duodenum, *H. pylori* may cause secretory diarrhoea by provoking inflammation, altering enzymes of the brush border, disrupting normal absorptive processes and injuring the villus surface. Thus, *H. pylori* may stimulate intestinal fluid secretion by the crypt cells. It might be accompanied by inhibition of absorption of fluid by the villus cell.

Another possibility could be osmotic diarrhoea caused by the presence in the intestinal lumen of unabsorbed osmotically active solutes, particularly carbohydrates. One speculation is that disacharidase deficiency and defects in carrier function, inhibit carbohydrate absorption in the upper small intestine. In addition, diarrhoea could result from abnormal intestinal motility, causing accelerated transit and reduced contact time between luminal contents and mucosal cells (Melvin et al, 1995). As abdominal pain is common in *H. pylori* infected children, it is possible that bacteria may induce a motility disturbance with rapid transit and decreased absorptive time for nutrients, resulting in diarrhoea.

If it is true that *H. pylori* infection due to reduced gastric acid secretion contributes to small bowel contamination, then bacterial overgrowth may also cause a diverse spectrum of absorptive disorders including steatorrhea, carbohydrate malabsorption, and vitamin deficiencies. These factors have been poorly investigated. This possible hypothesis, however, has not found enthusiastic support among researchers in the area of nutrition.

There are a number of mechanisms by which small bowel colonisation may lead to malabsorption. The most common clinical feature is malabsorption of fat accompanied with malabsorption of water-soluble vitamins. Anaerobic bacteria, such as bacteroides, anaerobic *Lactobacilli*, and *Clostridia* are able to deconjugate bile salts by hydrolysis (Hill and Drasar, 1968).

This process is known to impair adequate micelle formation, leading to fat malabsorption and steatorrhea (Dawson and Isselbacher, 1960).

Since impaired micelle formation causes an adverse change in intestinal transit time and consequently the formation of insoluble soaps, it may also affect the absorption of water-soluble nutrients and minerals (Murphy and Signer, 1974). An *in vitro* study reports that, presence of unconjugated bile salts may induce carbohydrate malabsorption by inhibiting intestinal active sugar transport (Gracey et al, 1971). This proposed mechanism, however, remains controversial. Another study of infants observed that bacterial overgrowth followed the monosaccharide intolerance diagnosed (Kilby et al, 1977).

Small bowel bacterial overgrowth is also associated with megaloblastic anaemia that occurs with deficiency of vitamin B_{12}. Malabsorption of the vitamin occurs by bacterial binding of the vitamin B_{12}-intrinsic factor complex, thereby preventing vitamin B_{12} absorption in the distal ileum (Giannella, et al 1971; King et al, 1979). Deficiencies of other vitamins apparently do not occur. This might be justified by the fact that intestinal bacteria are able to synthesise vitamin K, riboflavin, nicotinic acid and biotin (Donaldson, 1964). As with other bacteria, *H. pylori* might be competing for vitamins and other micronutrients present in the intestinal lumen of the host and contribute to nutrient malabsorption by causing physical interference and possibly of impairment enzymatic activity, such as inhibition of tryptic and lipase activity. The relative importance of these proposed mechanisms in *H. pylori* infection will remain uncertain until there is new evidence.

Normal gastric acid production is necessary for the absorption of calcium across the gut wall in ionized form (Schachter et al, 1960). It is believed that defective absorption may occur in hypo and achlorhydric subjects, especially in elderly women (Ivanovich et al, 1967; Recker, 1985). This might be important in *H. pylori* infected children because of the link between calcium deficiency and poor bone development. However, there are no data on growth retardation and malabsorption of calcium due to *H. pylori* infection-related achlorhydria.

Iron deficiency anaemia accompanies most nutritional deficiencies and can be caused by or aggravated by malabsorption due to parasites or bacterial infections. An *in vitro* study reports that *H. pylori* was able to obtain iron through the human lactoferrin receptor system and to have full growth in media lacking other iron sources, but supported by human lactoferrin (Husson et al, 1993). It is well established that the ability of pathogenic microorganisms to acquire iron from hosts is also an important virulence factor (Bullen et al, 1978; Weinberg & Weinberg, 1995). Most bacterial pathogens cannot replicate without acquiring iron from their hosts. Since the iron acquisition system of *H. pylori* may play a critical role in host-bacteria relations during the infection, it could cause an impact on the nutritional status of children.

Since gastric and intestinal epithelium are damaged in *H. pylori* infection, it may allow direct losses of protein through a mucosa particularly permeable to plasma protein. In addition, enzymatic activity may be decreased, affecting nutrient digestion in the intestinal lumen and causing faecal loss. Despite these interpretations, protein loss possibly associated with *H. pylori* infection in children has only been reported by Hill et al. (1987), hence, further studies are essential to verify this hypothesis.

The normal intestinal epithelium determines the nutritional and hydration status in humans, by absorbing nutrients, as well as balancing electrolytes and water in both directions across the mucosa. If it is true that *H.pylori* infection by different mechanisms causes diarrhoea, then it might only be a symptom of injured mucosa. Therefore, persistent lesions or further injuries by absorption of food toxins, foreign proteins or bacteria, may delay healing and predispose the individual to frequent episodes of diarrhoea and malabsorption of nutrients in childhood, leading to malnutrition.

Surprisingly, mucosal injury in the small intestine is responsible for nearly 5 million deaths worldwide and is most common in developing countries, where there is a pre-existence of malnutrition. In addition, diarrhoea with associated malnutrition is probably the most common cause of death among young children worldwide. It is well accepted that, episodes of diarrhoea are responsible for progressive deterioration of nutritional status, particularly, persistent diarrhoea which can cause deterioration of nutritional status, leading to poor health (Martorell et al, 1975; Mata et al, 1977; Martínez and Chávez, 1979). Hence, studies of the relation between *H. pylori* infection, injured mucosa, gastric acidity, and small bowel overgrowth in children with chronic diarrhoea are advised.

As in *H. pylori* infection, a reduced gastric acid secretion is well documented in malnutrition; which seems not to change significantly with nutritional rehabilitation (Gilman et al, 1988). However, factors associated with the decrease in gastric acid output in malnourished children are unclear (Gracey et al, 1977; Gilman et al, 1988). Malnutrition also produces mucosal atrophy and poor intestinal absorption through a variety of different mechanisms (James, 1970; Kumar et al, 1971; Behrens et al, 1987).

A plausible explanation could be that *H. pylori* infections are masked by misdiagnosis. Certainly, *H. pylori* infection may lead to malabsorption of nutrients and malnutrition by impairment of the gastrointestinal mucosal barrier.

4.2. Mechanisms of reduced food intake

H. pylori infection must also suppress the nutritional status in children through the immune response. As pointed out before, *H. pylori* secretes several factors, including potent neutrophil chemotaxins and substances capable of activing peripheral blood monocytes to produce the cytokines interleukin 1ß, tumour necrosis factor α (TNF-α), and IL-6, and induce secretion of mucosal IL-8. When there is an intestinal inflammatory response, interleukin-1 and TNF-α appear to be responsible for causing delayed gastric emptying, anorexia, diarrhoea and weight loss. It has been also reported that, interleukin-1 and TNF-α suppresses the food intake by altering the firing rates of glucose-sensitive neurones in the lateral hypothalamus (Plata-Salaman, et al, 1988).

Further, experimental studies have shown weight loss and delayed gastric emptying after systemic administration of interleukin-1 or tumour necrosis factor (Otterness et al, 1988; McHugh et al, 1993). It is clear that interleukin-1 and TNF-α are important contributors to alter gastrointestinal motility and decrease food intake during inflammation, but prostaglandins, corticotropin-releasing hormone and platelet activating factor may secondarily mediate these effects. A greater knowledge of nutrition-cytokine interactions in *H. pylori* infection may be needed to explain the role of bacteria in the pathogenesis of child malnutrition.

H. pylori infection could also affect nutritional status through systemic events that alter the control of food intake. Structural and functional integrity of the gastric and intestinal mucosa is disrupted by *H. pylori* infection, which, as discussed probably lead to impaired digestion and absorption. Abdominal cramps are common in *H. pylori* infected children, and may induce a motility disturbance with rapid transit. Each and all of these effects are considered to serve as signals for the modulation of food intake (Crompton, 1984). Thus, *H. pylori* infected children could have a reduced food intake due mainly to the effect of changed motility pattern. Since the anorexia is suspected to be a vital factor in weight loss or failure in weight gain in parasitic disease, *H. pylori* infection may not be ruled out. Accurate quantitative information about food intake in *H. pylori* infection is needed before its effects on nutritional status in children are understood.

In synthesis, one might speculate that *H. pylori* might be causing a threatening vicious cycle between damaged mucosa and malnutrition in infected children (Figure 4). The infection may cause deterioration of nutritional status in children, affecting dietary intake, nutrient absorption, and metabolic processes directly through the gastrointestinal tract (Figure 5). Hence, evidence is required concerning the role of *H. pylori* in the pathogenesis of diarrhoea associated with injured mucosa, malabsorption, and possible growth failure exerted through suppression of the gastric acid barrier and through the intestinal inflammatory response.

Despite impressive data on prevalence, little is known about *H. pylori* infection in the general child population with no gastrointestinal symptoms. It is obvious that the pathogenic role of the bacterium needs to be studied, because no clear-cut association can be observed between infected children and symptoms.

Figure 4. A simplified model of the way in which *H. pylori* may lead to adverse effects on nutrition

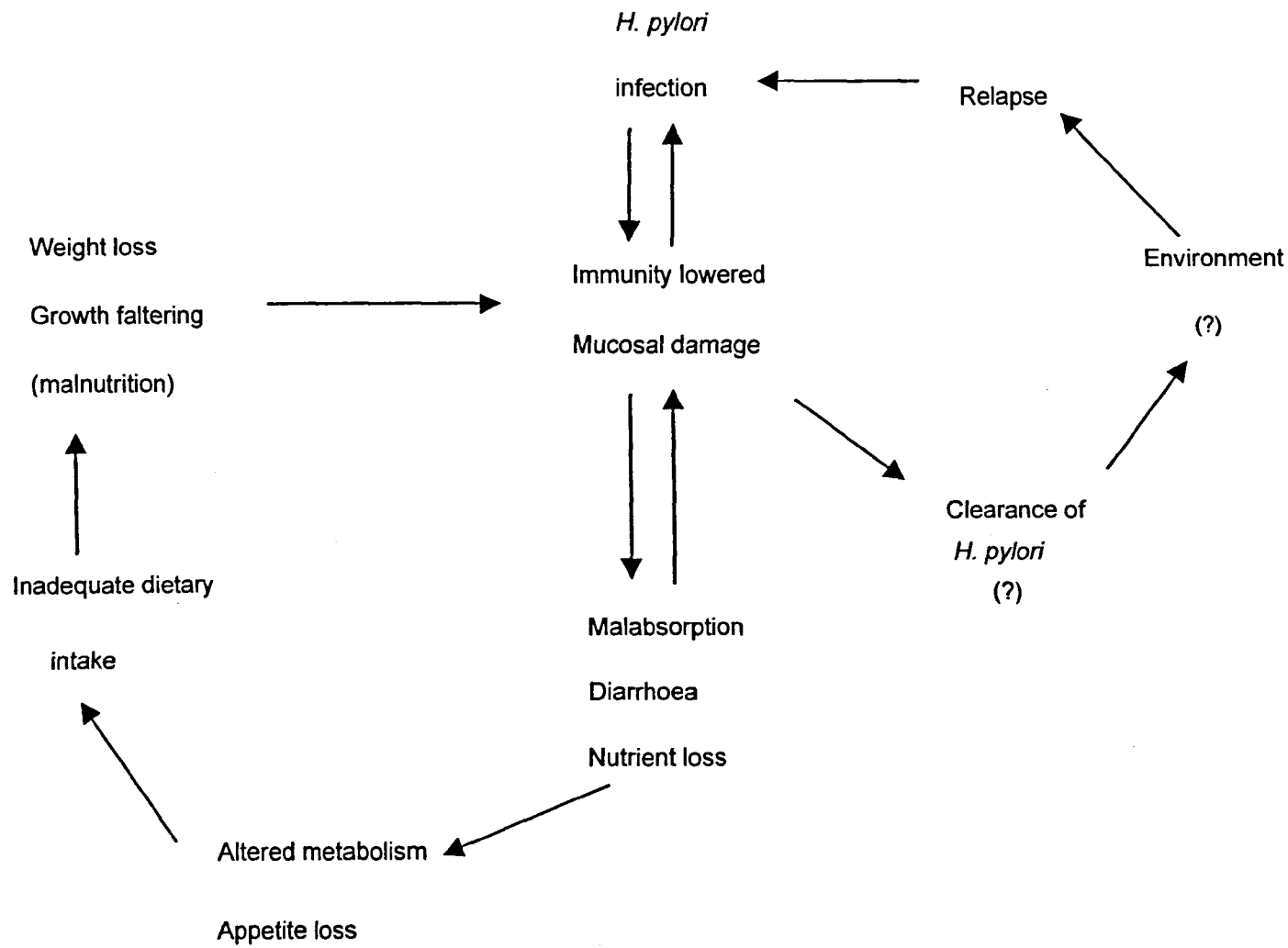
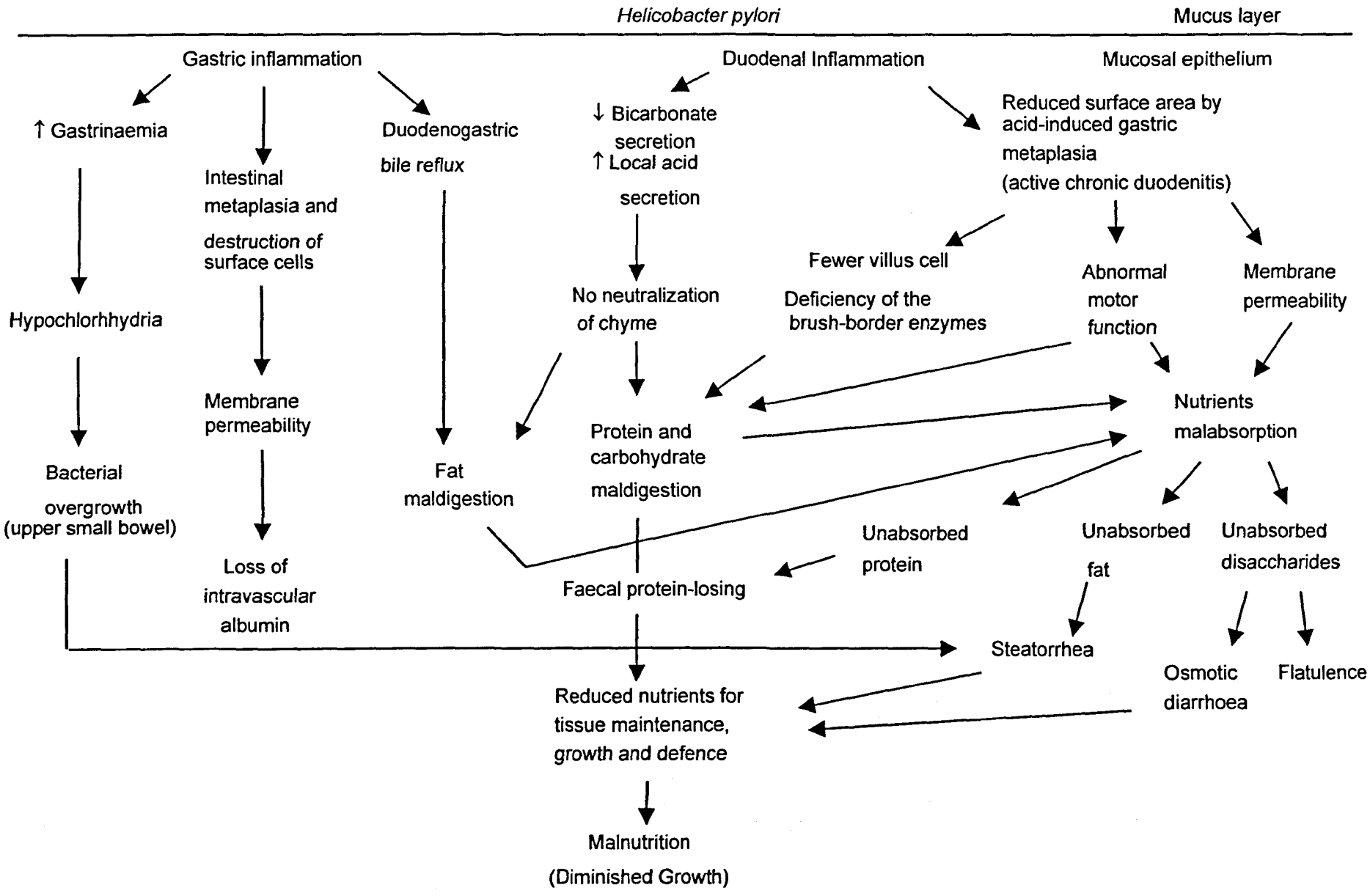


Figure 5. *H. pylori* events leading to malabsorption, diarrhoea and malnutrition



It is important to look at children with pathologies that are possibly masked by malnutrition. Consequently, the role that *H. pylori* may have in causing diarrhoea, malabsorption, and growth failure of children is important. Children are an excellent study population for *H. pylori* infection since confounding variables such as smoking, alcohol, and drugs, are not present.

To conclude, it is important to determine what risk factors, both in the individual and within households, play a role in the early acquisition of infection; whether infection affects nutrient intakes and their absorption; and which intervention strategies are suitable to prevent the spread of this infection, particularly among children. The impact on public health in developing countries of preventing this illness would be great. Additionally, the prevention of *H. pylori*-related morbidity and mortality would have a positive impact on the economies of developing countries.

CHAPTER II

STUDY PROTOCOL

1.- Objectives of Study

The general aim of this study was to estimate the prevalence of *H. pylori* infection among school children aged 9 and 10 years in Mexico and identify the potential risk factors that predispose to this infection in the community; as well as estimate the effects of this infection on growth in these children.

The specific objectives of the study were:

a) To determine the prevalence of *H. pylori* infection in children of both sexes from an urban neighbourhood of Hermosillo city, Sonora, Mexico.

b) To investigate the strength of the association between *H. pylori* infection and risk factors that may predispose children to infection in the community, including socio-economic status, living conditions and other related factors.

c) To estimate the effects on growth in school-age children resulting from exposure to *H. pylori* infection, while accounting for other factors that may have a negative influence on growth, such as energy, protein and iron intakes; as well as the presence of iron-deficiency anaemia and/or intestinal parasites.

d) To generate rationale for new studies and new preventive hypothesis, and to help develop and propose intervention strategies to deal with *H. pylori* infection among children aged 9 and 10 years in México.

2.- Study Setting

The study was conducted in 1997 and 1998 in the poorest socio-economic sectors of the city of Hermosillo, Sonora, Mexico. This city is located in the Northwest of the Country, on the coastal plain of the desert and lies 230 meters above sea level (Appendix A). Hermosillo is a city of about 583,320 inhabitants with 90% urban population. It was selected as the study setting because the true prevalence of *H. pylori* in children is unknown for this population and the city well is known to the Principal Investigator (P.I.), favouring the advantages of logistics, administration and communication to ensure the success of study.

Mexico is the only developing country belonging to the Northern region of the American continent. It has approximately 98 million inhabitants. As any developing country, Mexico has developed unevenly over the last thirty years in terms of social changes and industrial growth. Mass urbanisation has resulted in marginal groups, also found in poor rural communities. In spite of improvements in nutrition and health, severe and moderate malnutrition continues to affect approximately one in four Mexicans. It is estimated that one in two children aged between 1 and 5 years (the age group for which screening is more regular) may be malnourished.

3.- Study Design

3.1. Type of research design

The study employed a cross-sectional design (descriptive study) based on a census, including single observations at one point in time.

The study location was an urban neighbourhood within the city of Hermosillo, Sonora, Mexico. This study included children aged between their 9th and 11th birthday attending two urban schools with mainly children from poorer socio-economic households. The main outcomes of interest were infection with *H. pylori* and growth in children.

3.2. Sample size and sampling design

Assuming an expected infection prevalence of 40% (± 7.50% as the worst acceptable) and using a confidence level of 95%, the finite population-adjusted sample size required was of 164 children plus 10% to allow for losses and other contingencies. Hence, 180 children aged 9 and 10 years were selected. A stratified random sampling technique was used to achieve a more efficient estimation of *H. pylori* infection prevalence. The sampling frame was divided into groups by age and sex, thereafter; a simple random sample was then selected, in order to ensure that the proportions in each group perfectly reflect the proportions in the total population. Because *H. pylori* infection might be related to sex, sampling error was minimised by stratifying according to the corresponding age-sex distribution at the schools (48% boys and 52% girls).

4. Methodology

4.1. Recruitment

In order to select the study population, a census was conducted in eleven public primary schools from the poorest socio-economic sectors of the city of Hermosillo, Sonora. Two of these schools were chosen for their size and location (access and level of neighbourhood safety).

The study population was randomly selected by using the current registry of these two schools (Vicente Lombardo and Gabriela Mistral). The schools shared the same physical premises – the former using them in the morning and the latter in the afternoon.

The study was completed on 178 children aged 9 and 10 years (eighty-six boys and ninety-two girls) because two failed to keep all appointments and failed to deliver faecal samples. Where children of the same family fell into the random selection the next child on the school register was chosen as a replacement in order to prevent prevalence bias by known familial clustering of *H. pylori* infection. Where children and/or their parents refused to take part in the study, the same replacement method was applied.

More than 300 homes were visited initially in order to recruit the required sample size. These visits served to locate the children, check their date of birth (at last birthday) and locate replacement children. Accurate ages were recorded with the use of birth certificates. The purpose of the study was explained and informed written consent was obtained from the parents before children were enrolled.

4.2. Data collection

The reproducibility of instruments, procedures and methods of data collection were pre-tested (pilot study) on seven children who did not form part of the final study. During the pilot study, two field workers/laboratory assistants were trained in interviewing techniques (including 24-hour recall method for dietary intake), in the use of a precoded questionnaire, in the use of ^{13}C-Urea breath test for assessing *H. pylori* status, in the use of Kato-Katz procedure for faecal examination, in the procedure of haemoglobin measurement, and in the taking of anthropometric measurements.

Training of the two assistants (qualified senior medical laboratory officers) took 3 months and the standardisation procedures were rigorous. The same trained assistants participated in the main study, although only the female assistant was involved in the recording of anthropometric measurements.

Participating children's homes were visited on several occasions, in order to carry out the data collection. Some of these homes were re-visited, when the study child and/or mother was absent at the time of the visit. During these visits, the mother or primary caregiver (the grandmother in most cases) of each child was interviewed and the structured questionnaires completed. There were questions relating to the socio-economic status such as education, household income and living conditions; demographic data such as the date and place of birth, sex and parents' occupation, marital status and number of family members. During the visits, particular attention was given to the nature of the dwelling, its size, the type of plumbing (water and drainage pipes), toilets, kitchen and the sleeping arrangements. The 24-hour dietary recall was also administered to children at their homes.

The children (n = 178) were subjected to the ^{13}C-Urea breath test for the diagnosis of *H. pylori* infection. The *H. pylori* tests on study children along with anthropometric measurements (weight and height) and haemoglobin measurements were carried out at the schools. Three fresh faecal samples were collected serially during home visits and brought to the laboratory for immediate examination. The day before the faecal specimens were collected, pre-labelled plastic containers and explanations for the requirements of the sample collection were provided by the team.

4.3. Quality control

Questionnaires were pre-coded. To ensure data quality, the P.I. repeated 10% of home visits (chosen randomly). Weekly meetings with the field workers were held to review progress and resolve any unforeseen problems. Data records were checked for consistency and code regularly by assistants and P.I. Data entry was verified by entering the data twice and cross-checking

4.4. Methods of measurement of variables

a) Determination of *H. pylori* status

The presence of *H. pylori* was determined by the safe non-invasive ^{13}C-urea breath test as an accurate diagnostic test. This test is based on the principle that orally administered ^{13}C-urea is hydrolysed to CO_2 and ammonia by the active *H. pylori* urease. Care was taken not to test any child within two weeks of ingesting oral antibiotics, antisecretory agents (H_2-blockers and acid pump inhibitors) and/or mucosal protective agents, in order to avoid false negatives.

In this study, the following protocol was observed:
The ^{13}C-urea breath test was carried out in the morning after an overnight fast (a minimum of 7 hours). Two baseline breath samples were taken before the ingestion of the 99% ^{13}C-urea (Tracer Technologies, Boston, USA). Subjects blew air through a straw into the end of a 10 ml vacutainer (Becton Dickinson, Rutherford, NJ, USA) which was immediately re-sealed by the sample taker. Thereafter, the subjects were given an ice cream to eat as the test meal and ten minutes later they ingested ^{13}C urea (2mg/kg dose) dissolved in 40-60 ml of bottled water in one gulp.

Thirty minutes after swallowing the ^{13}C urea, duplicate samples were collected in 10 ml vacutainer similar to the baseline sample collection (Eggers et al, 1990).

The ratio of urea-derived $^{13}CO_2$ to $^{12}CO_2$ in the expired air samples was measured by automated gas-isotope-ratio mass spectrometry at the Nutrition Research Centre of St John's Medical College in Bangalore, India. The results obtained by this technique were expressed as excess δ ^{13} CO_2, excretion per thousand (δ ‰). A breath test with an excess over baseline of $\geq$ 5ml was regarded as a positive indication of *H. pylori* infection. A cut-off point of 5 in the difference in ratio was used for children, as recommended by Eggers et al (1990) and Logan et al (1991). This enrichment level, which is 120-fold greater than analytical variance and 20-fold greater than metabolic variations in $^{13}CO_2$, corresponds to the minimum adult level which is always associated with endoscopically proven *H. pylori* infection.

b) Anthropometry

Growth is a term, which is taken to mean an increase in the size of the body (usually in weight or height) over time. Here, anthropometric measurements such as height and weight were used as reliable descriptive indicators of variability of body size and linear growth due to their non-invasive nature and the relative ease with which they are taken. The general protocol for obtaining anthropometric measurements in this study followed the techniques recommended by expert anthropometrists (Cameron, 1986).

After an overnight fast, children reported to the school where their body measurements were taken by the P.I. (an experienced Nutritionist) and the female trained assistant who weighed the boys and the girls respectively throughout the study.

Children were weighed naked on a SCA 203 Weylux 424 Scale (measuring to a precision of 50 g) and the measurement was recorded to the nearest 50 g.

The P.I. or the female assistant measured standing height barefoot on a portable stadiometer (Harpenden) and the measurement was recorded to the nearest 0.1 cm. Two measurements were taken for height and when the difference between two readings was less than 1 cm, the mean measurement was recorded. Weighing scale and stadiometer were calibrated against known weights and lengths on a daily basis and were found to be consistently accurate.

Raw measurements of height and weight were used to derive the anthropometric indices of weight-for-age, height-for-age and weight-for-height expressed as a number of standard deviations or Z-scores below or above the reference mean (USA National Center for Health Statistics reference). A cut-off of below – 2 Z-scores relative to the reference mean was taken as indicative of growth failure, since this value is statistically defined as being out of the normality range (WHO, 1995).

c) Dietary intake

Dietary information was collected through three non-consecutive diet recalls (24-hour dietary recall method) in children, in order to obtain an estimate of the usual food ingested (Wiehl, 1942). The 24-hour dietary recall method is completely open-ended and permits estimations based on a greater level of detail for the types and quantities of food representing usual intakes on a specific day in epidemiological studies. In addition, previous studies have shown the validity of this method for children in developing countries, when estimates of energy and selected nutrient intakes to within ±10% are required (Rutishauser et al, 1973; Ferguson et al, 1989; Ferguson et al, 1994).

The P.I. and the two previously trained research assistants collected the dietary data by means of an in-depth interview (Appendix B). Special attention was given to obtain information on eating/drinking practices both at home and in the school, types of food, food preparation methods, recipe ingredients, including information on sharing of food, foods purchased (brand name identification) and eaten outside the home.

Each interviewer collected one recall per child, in order to eliminate the interviewer effect on the recalled intakes. The diet recall was administered to every child in the presence of his/her mother or primary caregiver. They were asked to recall all foods and beverages consumed by the child during the 24 hours preceding the interview. The respondents were also prompted about snacks consumed, and other family members (the siblings in most cases) were encouraged to provide information when appropriate. The quantities of consumed foods were estimated in household measures typical of the region (spoon, cup and plate set), using lifelike models or photographs. The ingredients of cooked foods consumed were recorded. For some foods consumed by the children it was possible to weigh comparable foods in size and amount by using 5 kg Salter digital scales (accurate to within ± 5g).

In order to convert the amount of food consumed from foods weighed or household measures to equivalent gram weight estimates, a local conversion table was used (National Institute of Nutrition in Mexico). Food intake data were converted into nutrient intake by using the nutrient composition tables published by the National Institute of Nutrition in Mexico (1996) and data on the composition of foods typical of the region. Nutrients were calculated on software designed by and used in the Centro de Investigación en Alimentación y Desarrollo, A.C. (CIAD, A.C.). The two trained assistants were involved in the input of the data in the computer.

The three dietary recalls for each child were converted into energy and nutrients and then averaged to obtain a one day estimate of usual/habitual intake. The requirements for energy and nutrients of children are dependent on body size, age, sex and physical activity. In order to judge dietary adequacy for each child the mean dietary intake was expressed as a percentage of the estimated requirements (RDA, 1989) per day and per kg of body weight.

d) Iron deficiency anaemia

Total concentration of circulating haemoglobin was measured colorimetrically, as haemoglobin is the main pigment in the blood. For this a "HemoCue" (a simple portable battery-operated photometer) was used, calibrated against the hemiglobincyanide method (international reference) (Vanzetti, 1966). The "HemoCue" system is comparable to standard laboratory techniques for measurement of haemoglobin level (von Schenck et al, 1986; Hudson-Thomas et al, 1994) in the general population and in anaemic children (Cohen and Seidl-Friedman, 1988).

Capillary blood was obtained by the finger-prick method (without squeezing) using disposable blood lancets (Microlance, Becton-Dickinson, Rutherford, NJ) and collected in a micro-cuvette containing a quantity of dry chemicals, which convert haemoglobin to haemoglobinazide, it was measured then and there. Accuracy of estimations was ensured by meticulous measurement taking and by daily checks of the control cuvette. The simplicity of the procedure made it ideal for field testing as well as being an objective, effective and acceptable screening procedure. Children were determined to be anaemic if their haemoglobin concentrations were at or below 11.5 g/dl as proposed by INAGG, 1979.

Estimates of the prevalence of iron-deficiency anaemia were based on the number of anaemic children.

e) Enteric parasites

Children collected fresh faecal specimens (not contaminated with urine) in a clean, pre-labelled plastic container with a screw-cap lid. Fresh faecal samples were collected from the children at home early in the morning and submitted immediately to the laboratory. Three separate specimens were sequentially collected and examined in order to achieve an accurate diagnosis of enteric parasites. The identification of cysts, eggs or larvae in the stool samples was assumed to indicate the presence of living enteric infections in the children at the time of stool collection.

The laboratory assistants carried out the examinations by following exact procedures. A macroscopic examination and a microscopic examination of fresh faecal specimens via direct wet mount, following a thick smear technique and concentration procedure (to remove sample debris) were performed. Faecal specimens were first examined grossly to determine the consistency (hard, formed, loosed, or watery), colour, and presence of gross abnormalities such as adult worms, pus, mucus, or blood. The consistency of faeces and the presence of abnormalities such as mucus and/or blood were used as an indication of the types of potential enteric parasites present.

Faecal specimens were examined for helminth eggs using a modified Kato-Katz technique recommended by WHO (1991), using templates to measure ≅ 50 mg of stool and a cellophane coverslip soaked in glycerine-malachite green solution for at least 24 hours.

The detection of protozoan cysts, roundworm eggs and dwarf tapeworm eggs was done using the zinc sulphate flotation method, which is based on differences in specific gravity between the sample debris and enteric parasites (Faust et al, 1938). The zinc sulphate solution was constantly monitored and adjusted as necessary in order to ensure accurate results. The two laboratory assistants examined all slides for 15 minutes.

The wet mount cover slip and thick smear were systematically scanned (within 24 h) under dry power at 10X, 20X and 40X, and X100 magnification respectively. The power only was increased at the higher magnification when a suspicious object required further examination.

f) Socio-demographic data and socio-economic status data

A questionnaire was designed to obtain basic family socio-demographic data such as sex, age, place of birth, education level, occupation, marital status, number of family members and family income (Appendix C).

g) Living conditions data

A questionnaire was designed to obtain information regarding number of rooms/bedrooms in the house, whether the kitchen was in a separate room and the number of persons living in the house (Appendix D). Particular attention was paid to obtain information regarding whether the study child and his/her siblings had its/their own bed/bedroom, the sleeping arrangements (whether somebody slept in the kitchen), pet/animal ownership and the type of plumbing (water pipe and sewerage system) in the house.

A crowding index was determined to express the number of people per room living in the household (number of people in the household divided by the number of bedrooms in the home). Where the house had no separate bedrooms, the number of communal rooms used as a living room and kitchen was considered. Five classes of crowding were identified (I - V), with each class corresponding to the number of people per bedroom: Class I = ≤ 1; Class II = 2 people, Class III = 3 people; Class IV = 4-5 people; and Class V = ≥ 6 people.

5. Data Processing and Statistical Analysis

5.1. Descriptive methods

Standard descriptive statistical analysis was conducted to examine and describe the distribution of cases for each variable under study, including numerical summaries of data as well as measures of relationship between variables.

5.2. Inferential statistics to analyse association between variables and to estimate the effects on growth by exposure to *H. pylori* infection

a) Univariate and bivariate statistics

Estimates of the standard error and 95% confidence intervals of the means, proportions and difference between two means/proportions were calculated, in order to determine the certainty or precision of these estimates in the population studied and so as to make univariate inferences.

For quantitative one-variable cases, the parametric *Student's independent t* test and 95% confidence intervals were used for the comparison of two independent means, when data were normally distributed and the variances in the two groups were roughly equal. When the quantitative variable was not normally distributed, the Mann-Whitney test (non-parametric) was used as an alternative considered more robust for that situation. For binary qualitative one-variable cases, the test of significance of chi-squared (χ^2) test was applied for the comparison of differences between two independent proportions or percentages.

Explanatory bivariate analysis was conducted to examine the relationship between one response variable and other explanatory variables under study. Apparent associations between two qualitative variables were further examined to quantify the strength and nature of the relationship, using the Pearson χ^2 test of independence/with Yate's continuity correction or if the expected value in any of the cells was smaller than 5, a Fisher's exact probability test was used.

The apparent linear relationship between two quantitative variables was explored, using simple bivariate coefficient of Pearson's product moment correlation (r) analysis to measure the direction of bivariate relationships in numerical terms and as well as using the bivariate linear regression to predict one variable from the other. The size of the correlation coefficient was interpreted as defined by Cohen and Holliday (1982): below 0.19 is very low; 0.20 to 0.39 is low; 0.40 to 0.69 is modest; 0.70 to 0.89 is high; and 0.90 to 1 is very high. The coefficient of determination (squared correlation coefficient r^2) was used in order to give a better description of the size of the correlation. Partial correlations were used to describe the relationship between two variables after statistically controlling for the influence of one or of some set of additional variables.

Confidence intervals for the separate bivariate correlation coefficients were calculated to consider the sampling variation. Non-parametric estimates of correlation were obtained by the Spearman rank correlation test, when two variables were related to each other in a non-linear relationship.

One-way analysis of variance (ANOVA) with one qualitative explanatory variable taking on two or more levels was employed in the testing of differences between means for the quantitative response variable. Two-way analysis of variance (ANOVA) was used to examine the relationship/association of a quantitative response variable with more than one qualitative explanatory variable.

a) Multivariate methods

Because bivariate correlations between all pairs of variables under study could not reveal complex interrelationships among variables, multivariate statistical analysis was conducted (as judgement analysis) to examine these relationships simultaneously, while controlling for potential confounding factors. Although statistical models commonly used in causal epidemiologic analyses of non-experimental data do not allow for the establishment of causality, associations among variables can be well quantified in a statistical understanding. Here, hence, the use of such models was a rational and practical way to address the issue of risk factors, as well as estimating the effects on growth by exposure to *H. pylori* infection.

In the context of this study, the use of the word effect presupposes testing a hypothesis about the influence of predictor variables on one response variable.

The direct logistic regression (logit regression) technique was used when the response variable was dichotomous/binary and the explanatory variables were quantitative, categorical or a mixture of both. The strength of the relationship between a response variable and the explanatory variables was estimated calculating the coefficient of multiple correlation (r) and the square of the correlation coefficient (r^2). Due to the strong multicollinearity between some explanatory variables or highly redundant factors, only one of a set of such variables and those that appeared to have the strongest independent effects were retained in the full model. Multiple analysis of variance was employed to examine the simple main effects of each explanatory variable and possible interaction between variables in relation to response variable.

Although normality of the variables is not always required for analysis, normality of continuous explanatory variables was assessed by both statistical and graphical methods. An indicator or dummy variable was created when one qualitative explanatory variable had several categories (polytomous variable). The statistical significance of a relationship that was observed in the set of sample data is expressed in terms of probabilities. The cut-off level for statistical significance was set at $p \leq 0.05$. The data were analysed using the Statistical Package for the Social Sciences (SPSS Inc., 1997).

6.-. Ethical Considerations

This study received ethical clearance from the Ethic Committees of the London School of Hygiene & Tropical Medicine, Nestlé Foundation, Fundación Mexicana para la Salud, Capítulo Sonora, and the Centro de Investigación en Alimentación y Desarrollo.

Children's parents were invited to take part, and gave their written consent (Appendix E). The P.I. ensured that parents of study children understood as fully as possible the nature of study, the reasons for its undertaking, and the possible benefit to health of children and their community as well as potential risks of participation in the study. The children themselves had the right to refuse and were at any time free to leave the study despite parental consent. All information collected was treated confidentially and was only available to individuals who had been approved by the P.I. and the parents of the participants.

A report with the study results for each child was written up and was given to all the parents in their own home (Appendix F). Children determined as being positive for *Helicobacter pylori* or enteric parasites were referred to a health unit of the state government where they received free treatment. The overall results of the study were also communicated to the children's parents and teachers separately in meetings held at the primary school.

CHAPTER III

RESULTS

This chapter is subdivided into four sections. The first section describes the family socio-demographic characteristics and living conditions of the study children. The second section analyses the prevalence of *H. pylori* infection between the study children in relation to associated risk factors within the community. The third section evaluates whether there is any significant relation between the presence of *H. pylori* infection and growth on the study children. The last section presents a report of the relationship of factors that might modify the effects of *H. pylori* infection on growth in the study children.

1. Family Socio-demographic Characteristics and Living Conditions

Table 4 shows the basic socio-demographic characteristics of the parents of the study children. The median ages of both the mothers and fathers were very similar. The levels of education of the mothers and fathers also had a similar distribution. Only 31% of both mothers and fathers had no primary education.

Nearly half the fathers were unskilled and semi-skilled workers, while 7% (11 cases) were reported as unemployed. As shown in Table 1, mothers were mostly housewives and the rest (25.8%) worked as employers, helpers in houses or temporary/day labourers. The study children were mostly born in the urban setting (92.1%). In contrast, nearly half of the parents were born in a rural setting (Table 4).

Table 4. Basic socio-demographic characteristics of the parents of study children (Hermosillo, Sonora, 1997)

	Mother	Father
Characteristic		
Age (years)[a]	35.4 ± 6.5	38.4 ± 8.4
Educational level:		
No primary education	31.4 %	31.7 %
Primary education	34.4 %	31.7 %
Secondary education	26.5 %	29.3 %
Urban origin	54.4 %	44.8 %
House-wives	74.1 %	
Marital status:		
Married	57.8 %	
Cohabiting	27.5 %	
Occupational class:		
Unskilled	18.0 %[b]	44.8 %
Semiskilled	6.7 %[b]	46.7 %
Household income (per month)[cd]	196.3	

[a]Media
[b]Calculated solely from mothers in employment
[c]Median
[d]USA Dollars

The number of children per family ranged between 1 (only two cases) and 11 (one case) and with a median of 3 children per family, including the study children. Twenty-two percent of households had at least one relative living in the same house. The median family income was $196.3 USA Dollars per month (equivalent to $1,600.00 Mexican Pesos). It should be noted that the number of children per family and family income were not normally distributed. Therefore, the median is used as the ideal measure in describing the true picture of these variables for the study population.

In spite of the low average income, the children were not in a situation of extreme poverty. It would be better to say that the family income was sufficient to cover the cost of basic food items but not basic goods. Families were mostly Catholics (89%) and the rest were Protestant.

The majority of the houses had only a few rooms; ranging from one (12%), two (33%), three (34%), four (13%), five (8%) up to six (only one household), not including the kitchen. The number of bedrooms per house ranged from zero (12%), one (42%), two (39%), three (6%) up to 4 (only one household). In general, houses had walls (82%) and a roof (74%) constructed with concrete, but 22% and 4% had roofs made of corrugated zinc sheets and corrugated cardboard sheets with a tar coating, respectively. Almost half of the houses had a concrete floor (41%), 23% a tile floor and 24% had a dirt floor. A kitchen as a separate room was reported by 70% of families.

Although most of the households (97%) had access to the main water supply; 62% had access solely to one tap in the yard and the remaining 35% had access to the piped water system inside the kitchen and the bathroom of the house. Only 84% of houses were connected to the sewerage system. Most of the houses (84%) had a toilet fully connected to water/sewerage systems and pit latrines were scarce and were only found in 28 houses (16%).

2. Prevalence of *H. pylori* Infection and Associated Potential Risk Factors

The overall prevalence rate of *H. pylori* infection for the children in Hermosillo as determined by this study was 47.1%.

The standard error calculated is 0.0014 (95% Confidence Interval (C.I.) 46.9 to 47.4), thereby allowing the deduction that the prevalence obtained is representative of the population studied. In this study, sampling error for the prevalence rate of *H. pylori* infection was reduced by an increase in the sample size and increased homogeneity of the elements sampled.

Table 5.- Prevalence rate of *H. pylori* infection in the study children

	Group of 9 years old % (n)	Group of 10 years old % (n)
Overall prevalence	44.2 (42)	50.6 (42)
Prevalence in boys: Within age group	31.8 (14)	57.1 (24)
Prevalence in girls: Within age group	54.9 (28)	43.9 (18)

Table 5 shows the prevalence of *H. pylori* infection within each age group and by gender. The overall prevalence was found not to be significantly different in children of both age groups (Yate's χ^2 = 0.492; p = 0.483). The overall prevalence of infection was also not significantly correlated with gender (Yate's χ^2 = 0.392; p = 0.531). However, a statistically significant difference in the proportions of *H. pylori* positive boys between the two age groups (Yate's χ^2 = 4.608; p = 0.032) was observed, but not for the girls (Yate's χ^2 = 0.704; p = 0.401).

Also, there was a significant gender difference for the prevalence of *H. pylori* infection between boys and girls aged 9 years (Yate's $\chi^2 = 4.210$; $p = 0.040$) but not for those aged 10 years (Yate's $\chi^2 = 0.974$; $p = 0.324$), after controlling for age.

The overall prevalence rate of *H. pylori* infection was not significantly correlated with urban or rural-born children (Yate's $\chi^2 = 1.115$; $p = 0.291$). This may be explained by the fact that study children were mostly born in urban settings (92.1%). The prevalence of infection among children was not significantly associated with the child's birth place, after controlling for age (Fisher's χ^2 = can not be calculated - c.n.b.c.; $p \geq 0.084$) and gender (Fisher's χ^2 = c.n.b.c.; $p \geq 0.267$).

On the other hand, the overall prevalence of infection was strongly and significantly associated with the parent's birth place; rural-born mother (Yate's $\chi^2 = 4.812$; $p = 0.028$) and rural-born father (Yate's $\chi^2 = 4.764$; $p = 0.029$). In addition, *H. pylori* infection in boys aged 9 and 10 years was statistically associated with mother's birth place (Yate's $\chi^2 = 4.952$; $p = 0.026$) and in children aged 10 years of both genders with father's birth place (Yate's $\chi^2 = 5.895$; $p = 0.015$), after controlling for gender and age.

H. pylori infection was further tested according to living conditions. The proportions of children determined as positive for *H. pylori* infection in relation to the variables of living conditions are shown in Table 6. The factors that showed a strong statistical association were household crowding, the number of siblings, the sharing of bed by the study child and siblings, sleeping in the kitchen and type of main water supply. There was a linear relation between crowding index and prevalence of *H. pylori* infection. Children from the most crowded homes (≥ 2 persons per bedroom) were more likely to be infected than children from less crowded homes ($p = 0.025$).

Table 6 . Proportions of children in each class who were *H. pylori* positive in relation to the variables of living condition

Variable	% (n)	χ^2 value (Degrees of freedom)	p-value
*Crowding index:			
Class I = ≤ 1	0.0 (0)		
Class II = 2	32.6 (14)		
Class III = 3	55.6 (15)		
Class IV = 4-5	43.1 (25)		
Class V = ≥ 6	62.5 (30)	11.143 (4)	0.025 [a]
Number of siblings:			
0-1	15.6 (5)		
2-3	50.0 (49)		
4-5	61.9 (26)		
≥ 6	66.7 (4)	17.667 (3)	0.001 [a]
Study child had own bedroom:			
Yes	0.0 (0)		
No	47.5 (84)	c.n.b.c. (1)	1.000 [c]
Study child had own bed:			
Yes	21.1 (12)		
No	59.5 (72)	21.471 (1)	0.000 [b]
Siblings had own bed:			
Yes	20.0 (8)		
No	55.1 (76)	13.932 (1)	0.000 [b]
Main water supply:			
Inside house	34.9 (22)		
One tap outside house	53.9 (62)	5.154 (1)	0.023 [b]
Sewerage system:			
Yes	44.7 (67)		
No	60.7 (17)	1.837 (1)	0.175 [b]
Excreta disposal:			
Toilet	45.3 (68)		
Pit latrine	57.1 (16)	0.889 (1)	0.346 [b]
Person sleeping in kitchen:			
Yes	60.4 (29)		
No	42.3 (55)	3.915 (1)	0.042 [b]

* Number of people living in the household/the number of bedrooms in the home
[a] p-values derived from Pearson χ^2 test
[b] p-values derived from χ^2 test with Yate's continuity correction
[c] p-values derived from Fisher's exact test

There was a noticeable lack of statistical association with the sharing of a bedroom by the study child, the sewerage system and the type of excreta disposal. However, the apparent lack of association of *H. pylori* infection with the sharing of a bedroom may be explained by the fact that none of the *H. pylori* positive children had their own bedroom, rather than a real lack of association.

The association of *H. pylori* infection status with presence of pets in the home is shown in Table 7. Seventy-nine percent of the families had at least an animal at home. Dogs (62.3%), cats (34.2%) and birds (8.9%) were animal/pets most reported as living in or around the homes. Ten percent had hens (raised for food) that lived around their homes.

H. pylori infection was not statistically associated with the presence of animals at home ($p = 0.988$). However, when the kind of animal was taken in consideration, there was a significantly positive association between the presence of hens and *H. pylori* infection ($p = 0.046$). This may be because most study children with hens at home were infected (13 cases) compared with only 5 non-infected children. No statistical association was found for the other kinds of animal reported such as dogs, cats, birds, rabbits, turtles and ducks. The prevalence of *H. pylori* infection was not statistically associated to direct contact with indoor/outdoor animals for study children ($p = 0.099$) even after controlling for age (Yate's $\chi^2 = 2.812$; $p \geq 0.094$) and gender (Yate's $\chi^2 = 1.179$; $p \geq 0.278$).

Table 7. Proportions of *H. pylori* positive children in relation to animal ownership variable

Variable	% (n)	χ^2 value (Degrees of freedom)	p-value
Any animal ownership:			
Yes	46.8 (66)		
No	48.6 (18)	0.000 (1)	0.988 [a]
Dog:			
Yes	45.0 (50)		
No	50.7(34)	0.340 (1)	0.560 [a]
Cat:			
Yes	44.3 (27)		
No	48.7 (57)	0.166 (1)	0.684 [a]
Bird:			
Yes	37.5 (6)		
No	48.1 (78)	0.304 (1)	0.581 [a]
Hen:			
Yes	72.2 (13)		
No	44.4 (71)	3.979 (1)	0.046 [a]
Rabbit:			
Yes	62.5 (5)		
No	46.5 (79)	c.n.b.c. (1)	0.478 [b]
Turtle:			
Yes	57.1 (4)		
No	46.8 (80)	c.n.b.c. (1)	0.709 [b]
Duck:			
Yes	100.0 (1)		
No	46.9 (83)	c.n.b.c. (1)	0.472 [b]

[a] p-values derived from χ^2 test with Yate's continuity correction
[b] p-values derived from Fisher's exact test

When all of the explanatory variables that were significant in the bivariate analysis were fitted into a direct logistic regression model to control for any confounding factors, the same strong effect of father's birth place (rural setting), number of siblings (≥ 3 per family), type of main water supply (one tap in the yard) and the sharing of bed by the study child were seen as potential risk factors for acquiring the infection (Table 8). The test of the full direct logistic regression model of *H. pylori* status (positive) on these four explanatory variables, was statistically reliable (χ^2 (4, n = 178) = 38.541; p = 0.000); indicating that the predictors, as set, can reliably distinguish between *H. pylori* positive and negative. The model correctly predicted 75.5% of the *H. pylori* negative children and 60.7% of the positive; the overall correct prediction was 68.3%.

Table 8. Results of logistic regression analyses for each potential risk factor for *H. pylori* status in the statistical model

Risk factor	% *H. pylori* positive (n)	Regression coefficient	p-value	Adjusted odds ratio	95% C.I.
Father's birth place:					
Urban	38.5 (30)				
Rural	56.3 (54)	0.8203	0.018	2.274	1.15-4.48
Number of siblings:					
≤ 2	35.0 (35)				
≥ 3	62.8 (49)	0.7319	0.036	2.07	1.04-4.12
Main water supply:					
Inside the house	34.9 (22)				
Tap in the yard	53.9 (62)	0.6934	0.055	2.00	0.98-4.07
Study child had own bed:					
Yes	21.1 (12)				
No	59.5 (72)	– 1.4697	0.000	0.23	0.10-0.50

The contribution of these four explanatory variables in the model can be summarised thus: 4.6% and 1.4% of the variance in *H. pylori* positive is explained by the sharing of beds by the study child and the rural-born father respectively, but number of siblings and type of main water supply did not contribute (0%). However, the odds ratio of 0.23 for the sharing of beds showed little change in the likelihood of acquiring *H. pylori* infection on the basis of a one unit change in this predictor variable.

The apparent correlations between mother's birth place, household crowding, person sleeping in the kitchen and presence of hens in the homes with *H. pylori* infection are spurious because they did not contribute to the prediction of infection status. The sharing of beds by the siblings were not fitted into the logistic regression model because of its strong statistical correlation with the sharing of beds by the study children (Yate's $\chi^2 = 51.752$; $p = 0.000$).

3. *H. pylori* Status and Its Possible Effect on Growth

Height (cm) was normally distributed for the study children; with a mean of 135.20, median of 135.10 and a coefficient of variation of 4.7%. In contrast, weight (kg) was not normally distributed (slightly positively skewed and kurtosed); with a mean of 31.68, median of 30.37 and a coefficient of variation of 21.9% (Figure 6). It should be noted that some variables such as weight are not expected to be rigidly normally distributed in the populations. Height and weight, however, were highly correlated ($r = 0.703$, $p = 0.000$) as defined by Cohen and Holliday (1982). Since a linear relationship between height and weight was observed (Figure 7), there is a clear indication that Pearson's correlation coefficient as calculated is statistically reliable.

Figure 6.- Distribution curves of Z-scores of children

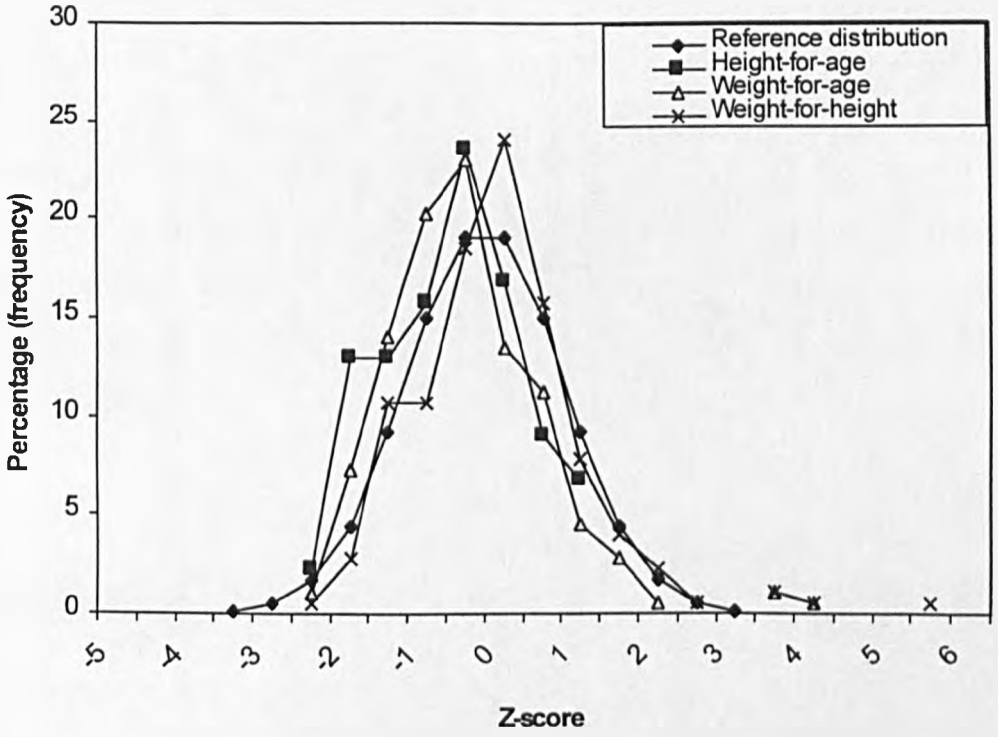


Figure 7. Scatterplot of weight with height

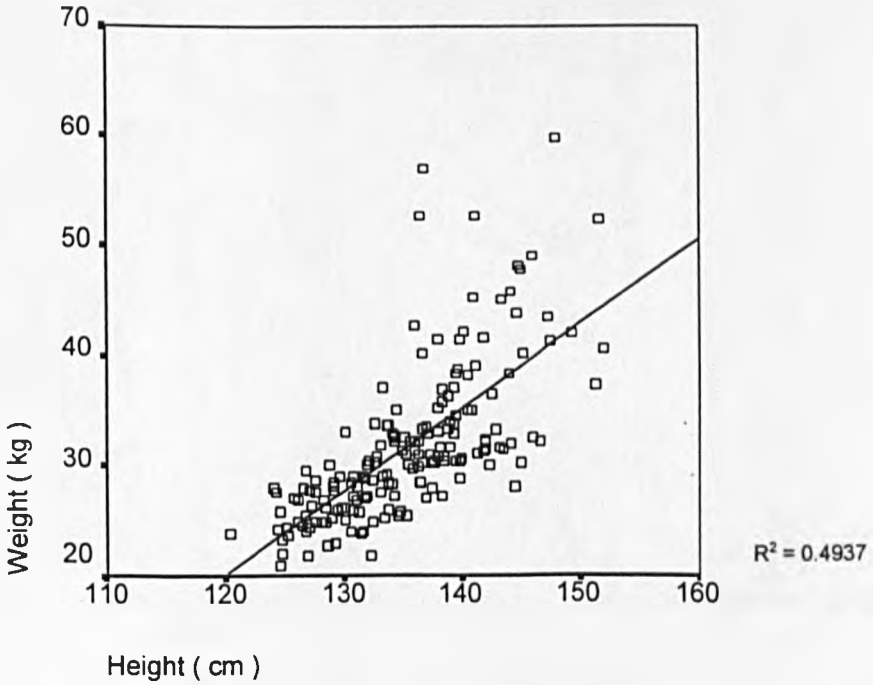


Table 9 shows the tabulation of anthropometric data for the study children described in terms of Z-scores and is compared with that of the reference population. As shown in the Figure 6 the curve of the weight-for-height distribution ran approximately parallel to, but slightly higher (positively kurtosed/skewed), than that of the reference population, whereas those of height-for-age and weight-for-age were normally distributed but slightly positively skewed and slightly kurtosed/skewed respectively. The overall prevalence rate of 2.2% for low height-for-age (– 2 S.D. of the reference) is similar to the reference population (2.3%).The overall prevalence rate for low weight-for-age and weight-for-height (– 2 S.D. of the reference) are 1.1% and 0.5% respectively.

Table 9.- Anthropometric data on the distribution of Z-scores in the study children

	Reference distribution (%)	Height-for-age (%)	Weight-for-age (%)	Weight-for-height (%)
Z-score range				
–3.49 to –3.0	0.1	0.0	0.0	0.0
–2.99 to –2.5	0.5	0.0	0.0	0.0
–2.49 to –2.0	1.7	2.2	1.1	0.5
–1.99 to –1.5	4.4	12.9	7.3	2.8
–1.49 to –1.0	9.2	12.9	14.0	10.6
–0.99 to –0.5	15.0	15.7	20.2	10.6
–0.49 to –0.0	19.1	23.5	23.0	18.5
0.01 to 0.5	19.1	16.8	13.4	24.1
0.51 to 1.0	15.0	8.9	11.2	15.7
1.01 to 1.5	9.2	6.7	4.4	7.8
1.51 to 2.0	4.4	0.0	2.8	3.9
2.01 to 2.5	1.7	0.0	0.5	2.2
2.51 to 3.0	0.5	0.0	0.0	0.5
3.01 to 3.5	0.1	0.0	0.0	0.0
3.51 to 4.0	0.0	0.0	1.1	1.1
4.01 to 4.5	0.0	0.0	0.5	0.5
4.51 to 5.0	0.0	0.0	0.0	0.0
5.01 to 5.5	0.0	0.0	0.0	0.0
5.51 to 6.0	0.0	0.0	0.0	0.5

The proportions of *H. pylori* positive children by anthropometric index did not show a marked increasing trend towards lower ranges of Z-scores values (Table 10). Height-for-age Z-scores were highly correlated with height (r = 0.84, p = 0.000), weight (r = 0.57, p = 0.000), weight-for age (r = 0.67, p = 0.000) but not weight-for-height (r = 0.07, p = 0.343). Weight-for-age Z-scores also were highly correlated with height (r = 0.59, p = 0.000), weight (r = 0.92, p = 0.000), height-for-age (r = 0.67, p = 0.000) and weight-for-height (r = 0.77, p = 0.000). Weight-for-height Z-scores were only highly correlated with weight (r = 0.74, p = 0.000) and weight-for-age (r = 0.77, p = 0.000).

Table 10.- Proportions of *H. pylori* positive and negative children in relation to anthropometric indices in terms of Z-scores values

	Height-for-age % (n)		Weight-for-age % (n)		Weight-for-height % (n)	
Z-score range	Positive	Negative	Positive	Negative	Positive	Negative
–3.49 to –3.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
–2.99 to –2.5	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
–2.49 to –2.0	0.5 (1)	1.6 (3)	0.5 (1)	0.5 (1)	0.5 (1)	0.0 (0)
–1.99 to –1.5	8.4 (15)	4.4 (8)	4.4 (8)	2.8 (5)	2.2 (4)	0.5 (1)
–1.49 to –1.0	7.3 (13)	5.6 (10)	6.7 (12)	7.3 (13)	4.4 (8)	6.1 (11)
–0.99 to –0.5	7.8 (14)	7.8 (14)	11.7 (21)	8.4 (15)	3.9 (7)	6.7 (12)
–0.49 to –0.0	9.5 (17)	14.0 (25)	8.9 (16)	14.0 (25)	7.8 (14)	10.6 (19)
0.01 to 0.5	7.8 (14)	8.9 (16)	6.1 (11)	7.3 (13)	14.0 (25)	10.1 (18)
0.51 to 1.0	4.4 (8)	4.4 (8)	5.0 (9)	6.1 (11)	5.0 (9)	10.6 (19)
1.01 to 1.5	1.1 (2)	5.6 (10)	1.6 (3)	2.8 (5)	5.0 (9)	2.8 (5)
1.51 to 2.0	0.0 (0)	0.0 (0)	0.5 (1)	2.2 (4)	1.1 (2)	2.8 (5)
2.01 to 2.5	0.0 (0)	0.0 (0)	0.5 (1)	0.0 (0)	1.1 (2)	1.1 (2)
2.51 to 3.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.5 (1)	0.0 (0)
3.01 to 3.5	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
3.51 to 4.0	0.0 (0)	0.0 (0)	0.5 (1)	0.5 (1)	0.5 (1)	0.5 (1)
4.01 to 4.5	0.0 (0)	0.0 (0)	0.5 (1)	0.0 (0)	0.5 (1)	0.0 (0)
4.51 to 5.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
5.01 to 5.5	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
5.51 to 6.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.5 (1)

When the association of *H. pylori* infection with low Z-scores values for anthropometric index was evaluated, a conventional clear cut-off point of – 1.5 S.D. was chosen as the most appropriate point to define the range from a health perspective rather than a statistically significant one. Since the study population showed a similar growth pattern to that of the reference values, this range might identify children who are more likely to fail to reach linear growth potential rather than simply determine those with shortness and thinness (– 2 S.D. of the reference).

Table 11.- Proportion of *H. pylori* positive children in relation to the anthropometric index in terms of Z-score ranges*

Anthropometric index	*H. pylori* positive % (n)	χ^2 value (Degrees of freedom)	p-value
Height-for-age:			
Below – 1.5	59.3 (16)		
Above – 1.49	45.0 (68)	1.333 (1)	0.248 [a]
Weight-for-age:			
Below – 1.5	60.0 (9)		
Above – 1.49	46.0 (75)	0.590 (1)	0.442 [a]
Weight-for-height:			
Below – 1.5	83.3 (5)		
Above – 1.49	45.9 (79)	c.n.b.c. (1)	0.102 [b]

[a] p-values derived from χ^2 test with Yate's continuity correction
[b] p-values derived from Fisher's exact test

There was a noticeable lack of statistical association between *H. pylori* infection and the number below the cut-off point of the three anthropometric indices (height-for-age, weight-for-age and weight-for-height) when tested by means of χ^2 test with Yate's continuity correction (Table 11), in spite of the higher cut-off point used (– 1.5 S.D). This apparent lack of association of *H. pylori* infection with the anthropometric indices may be explained by a low prevalence rate of low height-for-age and weight-for-age among the study children that was observed, rather than a real lack of association.

Screening anthropometric indices categorised by *H. pylori* status show that height-for-age Z-scores for positive (skewness = 0.206; kurtosis = – 0.789) and negative (skewness = – 0.133; kurtosis = – 0.501) groups follow a reasonably normal distribution. In contrast, the distributions of weight-for-age Z-scores for positive (skewness = 1.490; kurtosis = 4.644) and negative (skewness = 0.844; kurtosis = 1.735) groups and of weight-for-height Z-scores for positive (skewness = 0.784; kurtosis = 1.871) and negative (skewness = 1.533; kurtosis = 5.529) groups, are slightly skewed and kurtosed. Anthropometric indices in terms of Z-score values categorised by *H. pylori* status are not sufficiently skewed and kurtosed to make a substantial difference in the analysis, but the median may be a better measure to describe the variability of this data.

The Figures 8, 9 and 10 show the variability for Z-scores of height-for-age, weight-for-age and weight-for-height categorised by *H. pylori* status, including the median ("50th percentile"), the inter-quartile range (25th to 75th percentile), and the smallest and largest values. As can be seen in Figures 8, 9 and 10, the medians for height-for-age Z-scores (– 0.6444 vs – 0.3053) and weight-for-age Z-scores (– 0.4306 vs – 0.2256) were slightly lower for positive than negative children, but the opposite is true for the mean weight-for-height Z-scores (0.1981 vs 0.0937).

Figure 8. Height-for-age Z-scores by *H. pylori* status

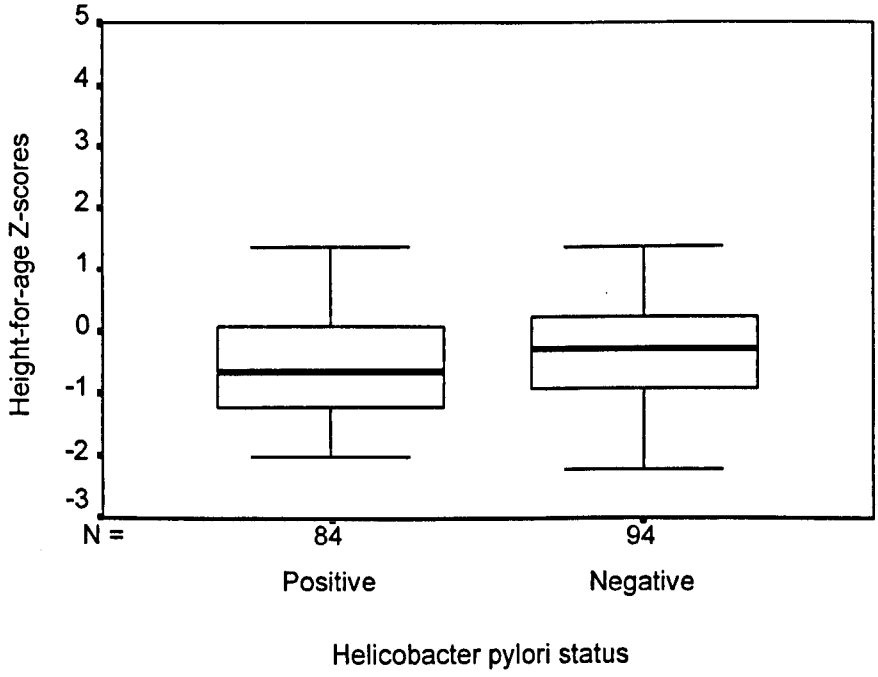


Figure 9. Weight-for-age Z-scores by *H. pylori* status

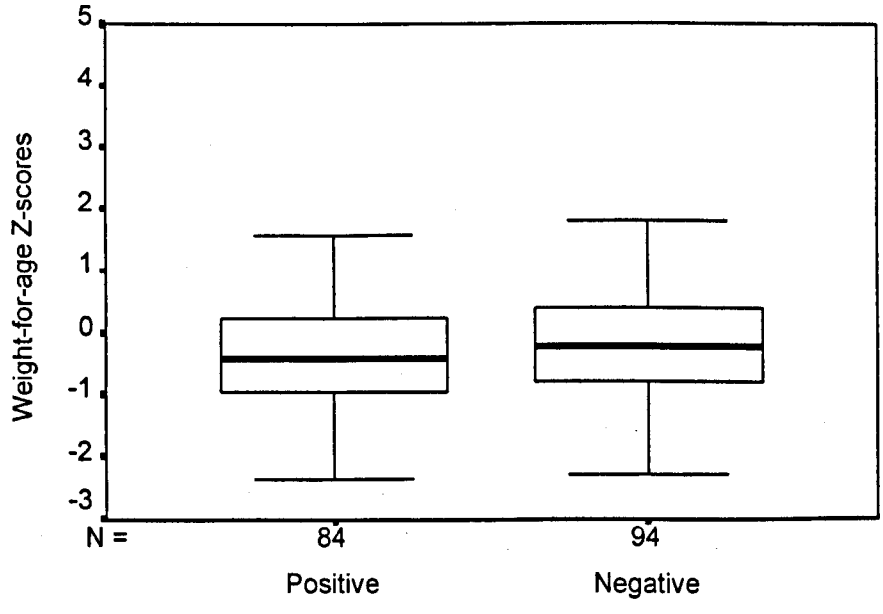
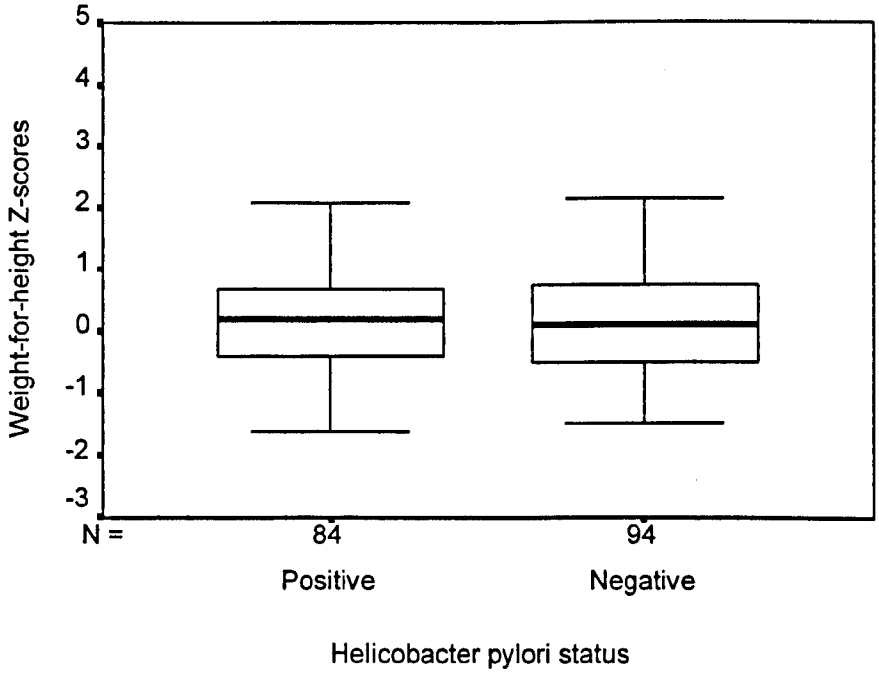


Figure 10.- Weight-for-height Z-scores by *H. pylori* status



To evaluate whether there is any significant relation between the presence of *H. pylori* infection and growth, positive and negative groups for height-for-age, weight-for-age and weight-for-height in terms of Z-score values were contrasted. There was a borderline significant difference in the mean ($p = 0.071$) height-for-age Z-scores between *H. pylori* positive and negative children. However, no difference in the mean weight-for-age and weight-for-height Z-scores was observed between infected and non-infected children (Table 12). Since the mean and median height-for-age Z-scores for both *H. pylori* positive and negative groups are numerically identical (Table 13) and the equality of variances between groups was satisfied (testing for normality), there is clear indication that the probability of the resulting *t* value is appropriate.

Table 12. Comparison of *H. pylori* positive and negative children by anthropometric index in terms of Z-score values*

Anthropometric index	*H. pylori* positive (n = 84)	*H. pylori* negative (n = 94)	F value[a]	p-value[a]	*t* value[b]	p-value[b]
Height-for-age	– 0.5481 ± 0.84	– 0.3122 ± 0.88	0.038	0.845	1.819	0.071
Weight-for-age	– 0.3086 ± 1.06	– 0.1408 ± 0.99	0.023	0.879	1.086	0.279
Weight-for-height	0.1597 ± 1.10	0.1868 ± 1.12	0.029	0.865	0.162	0.872

* Values are given as Mean± S.D.
[a] Levene's test for equality of variances
[b] Two tailed *t*-test for equality of means (equal variances assumed)

Table 13. Comparison of mean and median of anthropometric index in terms of Z-score values for *H. pylori* positive and negative children

Anthropometric index	*H. pylori* positive (n = 84)		*H. pylori* negative (n = 94)	
	Mean	Median	Mean	Median
Height-for-age Z-scores	– 0.5481	– 0.6444	– 0.3122	– 0.3053
Weight-for-age Z-scores	– 0.3086	– 0.4306	– 0.1408	– 0.2256
Weight-for-height Z-scores	0.1597	0.1981	0.1868	0.0937

The extent of the effect of the difference between the mean height-for-age Z-scores of the *H. pylori* positive and negative groups is 0.27, signifying a small effect for the paired and group *t* test as defined by Cohen (1988 and 1992). This small effect must be due to the small sample size "for this study population" which did not allow for a significant result.

A sample size of somewhere between 393 and 472 (from Cohen's tables) would be needed for each group positive and negative *H. pylori*, to detect such a small effect to a power of 0.80 (as a suitable value) in order to show a significant result when a real effect exists.

The extent of the effect of the differences between the mean weight-for-age and weight-for-height Z-scores of the *H. pylori* positive and negative groups is 0.16 and 0.02 respectively. This means there is no effect for the paired and group *t* test as defined by Cohen (1988 and 1992).

To examine the simple main effects and possible interaction of *H. pylori* infection and gender in relation to height-for-age, weight-for-age and weight-for-height in terms of Z-score values, the means of all groupings were compared, with the use of multiple analysis of variance (Table 14). Since anthropometric indices take into account height and weight differences related to age, age was not included as a factor in this analysis. A borderline significant main effect of *H. pylori* infection on height-for-age ($p = 0.062$) was detected but not for gender nor interaction of *H. pylori* infection by gender ($p = 0.080$).

The coefficient of determination (R^2) provides a measure of how much of the variation in the response variable is explained by explanatory variables. *H. pylori* infection and gender only accounted for 3.6% of the total variation in height-for-age Z-score (Table 14). There was a noticeable lack of significant effect of *H. pylori* infection on weight-for-age and weight-for-height and neither effect of gender nor interaction of *H. pylori* infection by gender (Table 14). Since the data did not violate the assumptions of the ANOVA and because the test has been shown to be fairly robust.

Table 14. Main effects and interaction of *H. pylori* infection and gender in relation to height-for-age, weight-for-age and weight-for-height Z-score values

Source*	ANOVA Sum of squares	Degrees of freedom	Mean square	F value	p-value	Observed power[a]
1.- Height-for-age						
*Corrected Model	4.803[b]	3	1.601	2.161	0.094	0.543
**H. pylori*	2.613	1	2.613	3.528	0.062	0.463
*Gender	0.011	1	0.011	0.016	0.901	0.052
**H. pylori*/gender	2.296	1	2.296	3.100	0.080	0.417
2.-Weight-for-age						
*Corrected Model	4.724[c]	3	1.575	1.499	0.217	0.391
**H. pylori*	1.326	1	1.326	1.262	0.263	0.201
*Gender	0.238	1	0.238	0.226	0.635	0.076
**H. pylori*/gender	3.120	1	3.120	2.970	0.087	0.403
3.- Weight-for-height	1.318[d]	3	0.439	0.351	0.788	0.118
*Corrected Model	0.037	1	0.037	0.030	0.862	0.053
**H. pylori*	0.172	1	0.172	0.138	0.711	0.066
*Gender	1.057	1	1.057	0.845	0.359	0.150
**H. pylori*/gender						

[a] Computed using $\alpha = 0.05$
[b] R squared = 0.036
[c] R squared = 0.025
[d] R squared = 0.006

4. Factors that may Modify the Effects of *H. pylori* Infection on Growth

4.1. Co-existing parasitic infections

Since parasitic infections are also known to affect nutritional status and growth of children, this study took a simultaneous look for the presence of these confounders. It was important to ascertain whether the prevalence data pointed to associations between *H. pylori* infection with any of the parasitic infections of public health importance, as well as whether the association was interactive or synergistic in relation to growth.

Table 15 shows the prevalence rate of intestinal parasitism in the study children. Although 39 study children (22%) were neither non-infected with enteric parasites nor *H. pylori* infection, most of the study children were found to harbour more than one infection at one time. Evidence of enteric parasites observed in the stools of *H. pylori* positive children (n = 84) was as follows: 27 (32%) had one parasite, 24 (28%) had two parasites, 10 (12%) had three parasites and 3 (3%) had four parasites.

Table 15. Prevalence rate by age for intestinal parasitism in the study children

	9 years old n = 95	10 years old n = 83	Overall prevalence for both age groups n = 178
Parasite genera			
I.- Protozoans:			
Endolimax nana	30.5%	37.3%	33.7% (60)
Entamoeba coli	24.2%	26.5%	25.2% (45)
Giardia lamblia	21.0%	10.8%	16.2% (29)
Iodamoeba buetschlii	0.0%	2.4%	1.1% (2)
Entamoeba histolytica	1.0%	1.0%	1.1% (2)
II.- Helminths:			
Hymenolepis nana	29.4%	36.1%	32.5% (58)
Ascaris lumbricoides	5.2%	7.2%	6.1% (11)
Hymenolepis diminuta	1.0%	0.0%	0.5% (1)
Trichuris trichiura	1.0%	0.0%	0.5% (1)

( ) Number of children with positive enteric parasite

The association of *H. pylori* infection with positive enteric parasites is shown in Table 16. *H. pylori* infection was found to be positively highly associated with *Hymenolepis nana* infection but there was a noticeable lack of association with other enteric parasitic infections.

Table 16. Proportions of *H. pylori* positive children in relation to the presence of intestinal parasitism

Parasite genera	% (n)	χ^2 value (Degrees of freedom)	p-value
I.- Protozoans:			
Endolimax nana			
Positive	51.7 (31)		
Negative	44.9 (53)	0.482 (1)	0.488 [a]
Entamoeba coli			
Positive	55.6 (25)		
Negative	44.4 (59)	1.271 (1)	0.259 [a]
Giardia lamblia			
Positive	48.3 (14)		
Negative	47.0 (70)	0.000 (1)	1.000 [a]
Iodamoeba buetschlii			
Positive	50.0 (1)		
Negative	47.2 (83)	c.n.b.c.	1.000 [b]
Entamoeba histolytica			
Positive	50.0 (1)		
Negative	47.2 (83)	c.n.b.c.	1.000 [b]
II.- Helminths:			
Hymenolepis nana			
Positive	65.5 (38)		
Negative	38.3 (46)	10.529	0.001 [a]
Ascaris lumbricoides			
Positive	54.5 (6)		
Negative	46.7 (78)	0.037	0.847 [a]
Hymenolepis diminuta			
Positive	0.0 (0)		
Negative	47.5 (84)	c.n.b.c.	1.000 [b]
Trichuris trichiura			
Positive	100 (1)		
Negative	46.9 (83)	c.n.b.c.	0.472 [b]

[a] p-values derived from χ^2 test with Yate's continuity correction
[b] p-values derived from Fisher's exact test

To evaluate whether any effect on growth was associated with coexisting enteric parasitic infections, children with positive and negative parasite for height-for-age, weight-for-age and weight-for-height Z scores were compared (Tables 17, 18 and 19).

There was a borderline significant difference in mean weight-for-age Z-scores between *Hymenolepis nana* positive and negative children ($p = 0.067$) but also a noticeable lack of significant difference for other enteric parasitic infections (Table 17). Although, the assumption was satisfied of equal variances between both *Hymenolepis nana* positive and negative groups, weight-for-age Z-scores in each group were not normally distributed in the study children, thereby, indicating that the probability of the resulting *t* value is not appropriate. In addition, no differences in the mean height-for-age and weight-for-height Z-scores were observed between infected and non-infected children with other enteric parasitic infections (Tables 18 and 19).

As mentioned above, *Hymenolepis nana* was found to be the only intestinal parasite positively highly associated with *H. pylori* infection. However, combinations between groups categorised by height-for-age Z-scores naturally occurred with unequal numbers of cases in each group and violated the assumption of normal distribution for ANOVA. As there are an unequal number of cases in each group, comparisons of means between groups give less reliable results and affect the overall p value (apparently significant). It was therefore not appropriate to examine the main effects and possible interaction of *H. pylori* infection, *Hymenolepis nana* and gender in relation to height-for-age Z-scores.

Table 17. Comparison of children with positive and negative enteric parasites by weight-for-age in terms of Z-score values*

Parasite genera	Positive	Negative	F value[a]	p-value[a]	t value	p-value
Endolimax nana	– 0.3667 ±0.85	– 0.1454 ± 1.10	1.133	0.289	– 1.359[b]	0.176[b]
Entamoeba coli	– 0.2874 ±1.04	– 0.1972 ± 1.02	0.193	0.661	– 0.507[b]	0.613[b]
Giardia lamblia	– 0.2107 ±0.86	– 0.2218 ±1.06	0.268	0.606	0.053[b]	0.958[b]
Iodamoeba buetschlii	– 0.9656 ±0.18	– 0.2115 ±1.03	1.676	0.197	–1.030[b]	0.304[b]
Entamoeba histolytica	– 0.3383 ±0.22	– 0.2186 ±1.03	1.550	0.215	– 0.163[b]	0.871[b]
Hymenolepis nana	– 0.4232 ±0.86	– 0.1218 ±1.08	0.592	0.443	– 1.844[b]	0.067[b]
Ascaris lumbricoides	0.1389 ±1.45	– 0.2436 ±0.99	1.568	0.212	1.195[b]	0.234[b]
Hymenolepis diminuta	– 0.4020 ± c.n.b.c.	– 0.2190 ±1.03	c.n.b.c.	c.n.b.c.	– 0.177[c]	0.860[c]
Trichuris trichiura	0.3356 ± c.n.b.c.	– 0.2231 ±1.03	c.n.b.c.	c.n.b.c.	0.540[c]	0.590[c]

* Values are given as Mean± S.D.
[a] Levene's test for equality of variances
[b] Two tailed *t*-test for equality of means (equal variances assumed)
[c] Two tailed *t*-test for equality of means (equal variances not assumed)

Table 18. Comparison of children with positive and negative enteric parasites by height-for-age in terms of Z-score values*

Parasite genera	Positive	Negative	F value[a]	p-value[a]	t value	p-value
Endolimax nana	– 0.4928 ±0.92	– 0.3883 ± 0.84	0.395	0.531	– 0.758[b]	0.450[b]
Entamoeba coli	– 0.4203 ±0.97	– 0.4246 ± 0.83	1.706	0.193	0.029[b]	0.977[b]
Giardia lamblia	– 0.4023 ±0.88	– 0.4277 ±0.86	0.026	0.871	0.143[b]	0.886[b]
Iodamoeba buetschlii	– 0.1309 ±0.91	– 0.4269 ±0.87	0.039	0.844	0.478[b]	0.633[b]
Entamoeba histolytica	– 0.8233 ±0.20	– 0.4190 ±0.87	2.633	0.106	– 0.653[b]	0.515[b]
Hymenolepis nana	– 0.5825 ±0.98	– 0.3467 ±0.79	6.533	0.011	– 1.706[c]	0.090[c]
Ascaris lumbricoides	– 0.2914 ±0.87	– 0.4322 ±0.87	0.002	0.967	0.519[b]	0.604[b]
Hymenolepis diminuta	– 0.2206 ± c.n.b.c.	– 0.4247 ±0.87	c.n.b.c.	c.n.b.c.	0.234[c]	0.816[c]
Trichuris trichiura	0.2576 ± c.n.b.c.	– 0.4274 ±0.87	c.n.b.c.	c.n.b.c.	0.785[c]	0.433[c]

–* Values are given as Mean± S.D.
[a] Levene's test for equality of variances
[b] Two tailed *t*-test for equality of means (equal variances assumed)
[c] Two tailed *t*-test for equality of means (equal variances not assumed)

Table 19. Comparison of children with positive and negative enteric parasites by weight-for-height in terms of Z-score values*

Parasite genera	Positive	Negative	F value[a]	p-value[a]	t value	p-value
Endolimax nana	0.0596 ± 1.00	0.2322 ± 1.16	0.050	0.824	– 0.978[b]	0.329[b]
Entamoeba coli	0.0782 ± 1.14	0.2083 ± 1.10	0.354	0.552	–0.705[b]	0.482[b]
Giardia lamblia	0.1705 ± 0.87	0.1747 ± 1.15	1.166	0.282	– 0.019[b]	0.985[b]
Iodamoeba buetschlii	– 0.7769 ± 0.69	0.1848 ± 1.11	0.365	0.546	– 1.218[b]	0.225[b]
Entamoeba histolytica	0.4078 ± 0.05	0.1714 ± 1.11	2.137	0.146	0.298[b]	0.766[b]
Hymenolepis nana	0.093 ± 0.94	0.2127 ± 1.18	0.184	0.669	– 0.667[b]	0.506[b]
Ascaris lumbricoides	0.6952 ± 1.90	0.1397 ± 1.03	3.254	0.073	1.612[b]	0.109[b]
Hymenolepis diminuta	– 0.3281 ± c.n.b.c.	0.1769 ± 1.11	c.n.b.c.	c.n.b.c.	– 0.452[c]	0.652[c]
Trichuris trichiura	0.2544 ± c.n.b.c.	0.1736 ± 1.11	c.n.b.c.	c.n.b.c.	0.072[c]	0.942[c]

* Values are given as Mean± S.D.
[a] Levene's test for equality of variances
[b] Two tailed *t*-test for equality of means (equal variances assumed)
[c] Two tailed *t*-test for equality of means (equal variances not assumed)

4.2. Dietary intake

Since inadequate nutrition, (especially deficiencies in energy, protein and iron intake) is just one of several causes of growth deficits in childhood, this study looked to evaluate the effect of these confounders at the same time.

Table 20. Descriptive statistics of energy and nutrient intake for the study children

	Mean	Median	Standard deviation	Skewness	Kurtosis	Coefficient of variation
I.- Energy:						
Per day (kcal)	1743	1725	451.21	0.747	1.024	25.88%
Percentage of RDA per kg of body weight	81	79	21.89	0.379	0.076	27.12%
Percentage of RDA per day	87	86	22.56	0.747	1.024	25.88%
II.- Protein:						
Per day (g)	52	49.89	15.73	0.609	0.370	30.19%
Percentage of RDA per kg of body weight	113	110	35.13	0.559	0.683	31.22%
Percentage of RDA per day	186	178	56.21	0.609	0.370	30.19%
III.- Iron:						
Per day (mg)	19	18	6.70	1.174	3.311	34.99%
Percentage of RDA per day	169	163	63.46	0.908	1.466	37.55%

RDA values used to calculate the results:
Energy = 2,000 kcal per day and 70 kcal per kg of body weight
Protein = 28 g per day and 1.5 g per kg of body weight
Iron = 10 mg for 9 years old boys and girls; 12 mg for 10 years old boys and 15mg for 10 years old girls

Table 20 provides descriptive statistics for the mean daily intakes of energy, protein and iron for the study children, expressed in three ways: mean intake per day, a percentage of corresponding recommended nutrient intake per day and per kg of body weight/day (calculated from age, gender and weight using Recommended Dietary Allowances, 1989). The coefficients of variation were calculated in order to express the variability of energy, protein and iron intake between the study children. As can be seen in Table 20, iron intake had the higher variability of the three.

Energy intake expressed as kcal per day and a percentage of recommended energy intakes were almost normally distributed but slightly kurtosed (Figures 11 and 12). However, when expressed as a percentage of recommended energy intake per kg of body weight/day the distribution was normal (Figure 13).

Figure 11. Histogram of energy intake per day

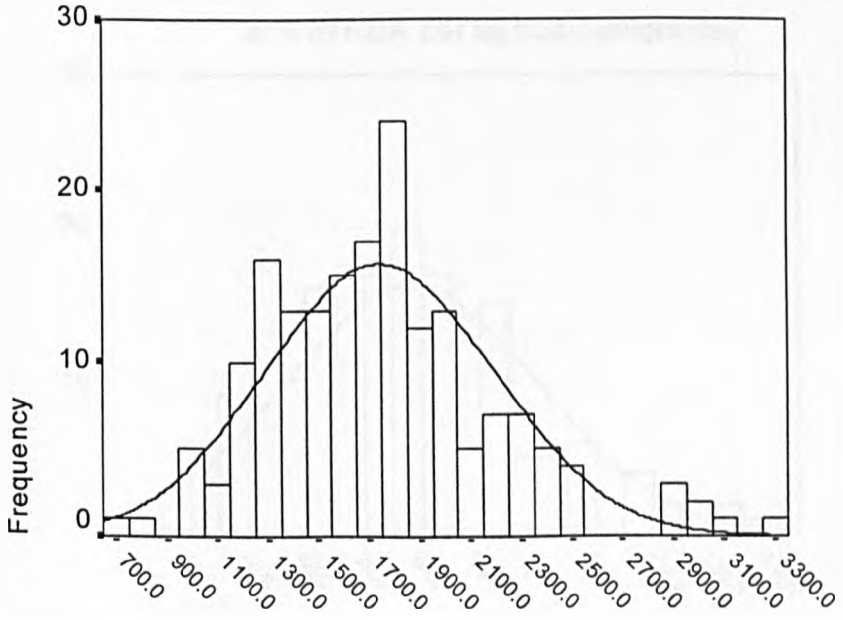


Figure 12. Histogram of energy intake expressed as a % of RDA per day

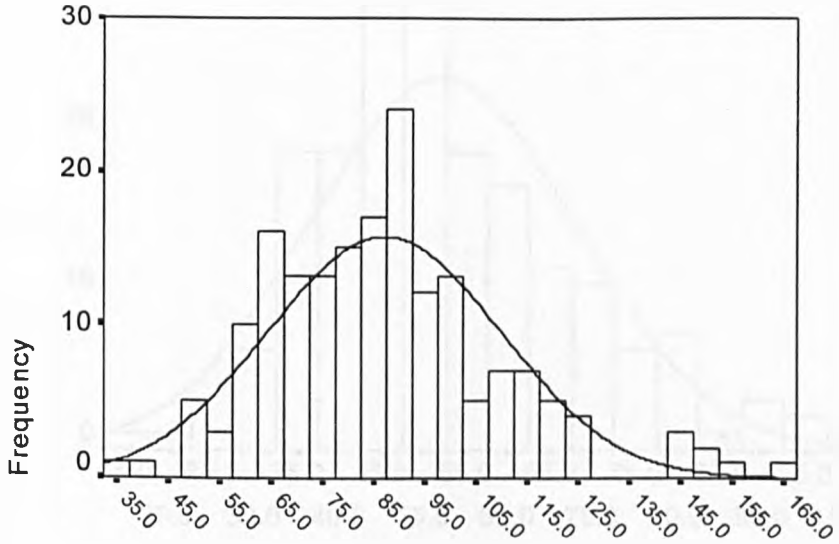


Figure 13. Histogram of energy intake expressed as a % of RDA per kg body weight/day

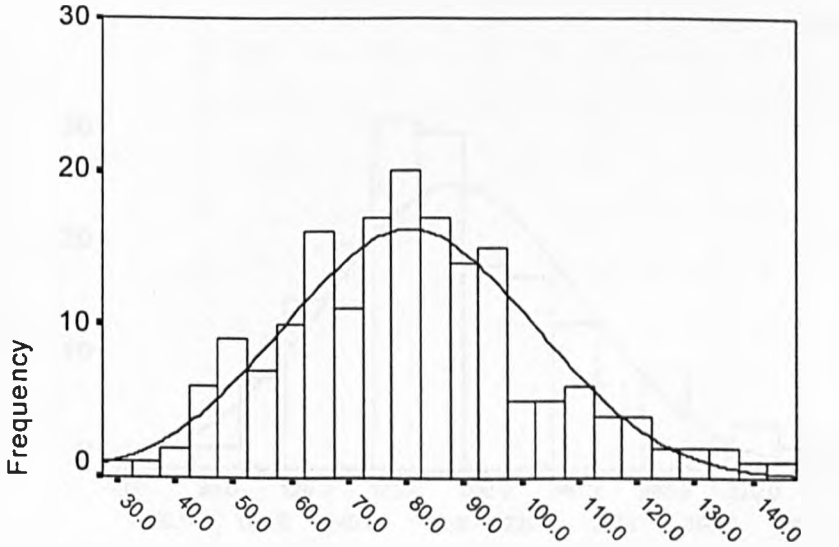


Figure 14. Histogram of protein intake per day

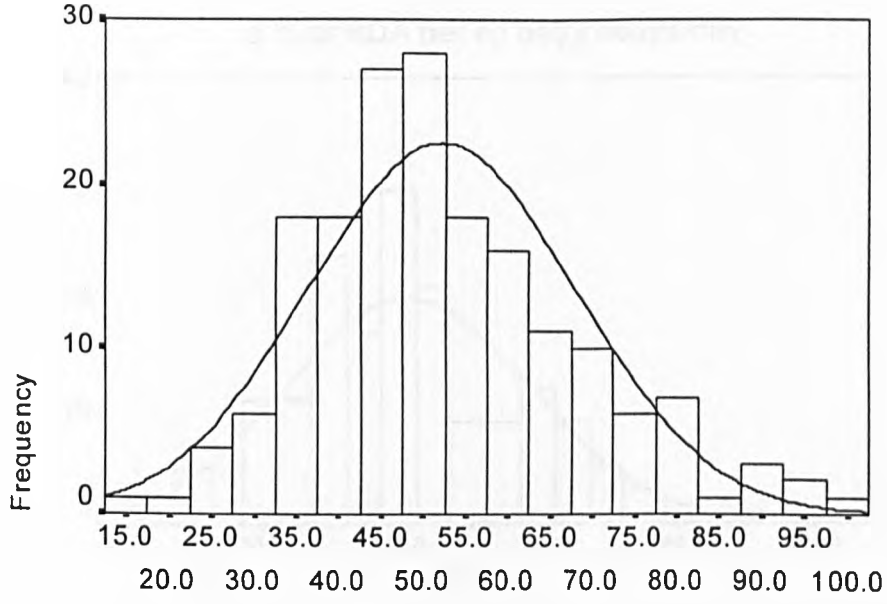


Figure 15. Histogram of protein intake expressed as a % of RDA per day

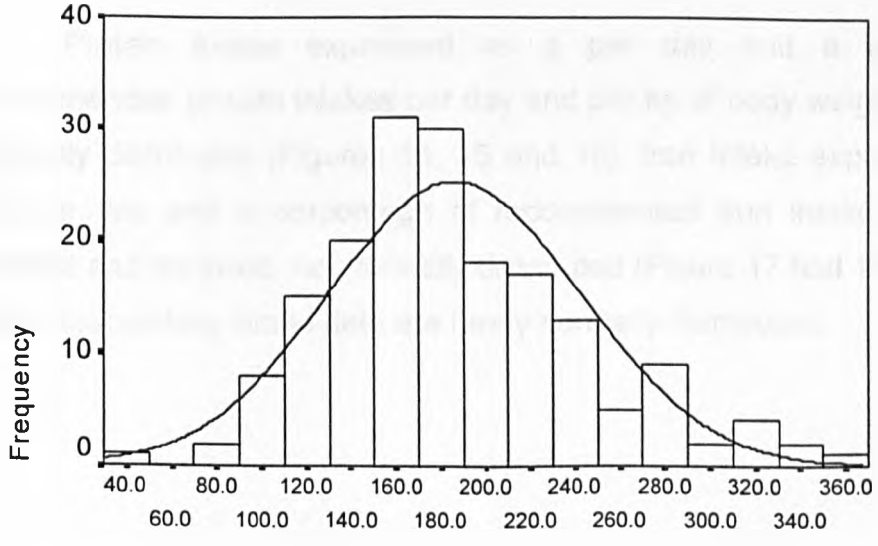
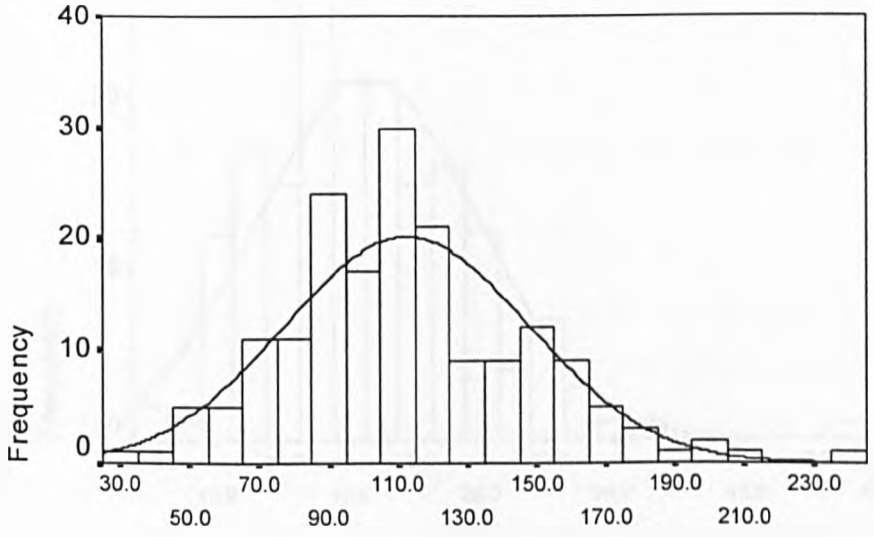


Figure 16. Histogram of protein intake expressed as a % of RDA per kg body weight/day



Protein intake expressed as g per day and a percentage of recommended protein intakes per day and per kg of body weight/day was not normally distributed (Figures 14, 15 and 16). Iron intake expressed as both mg per day and a percentage of recommended iron intake per day was skewed and kurtosed, not normally distributed (Figure 17 and 18). It should be noted that dietary intake data are rarely normally distributed.

Figure 17. Histogram of iron intake per day

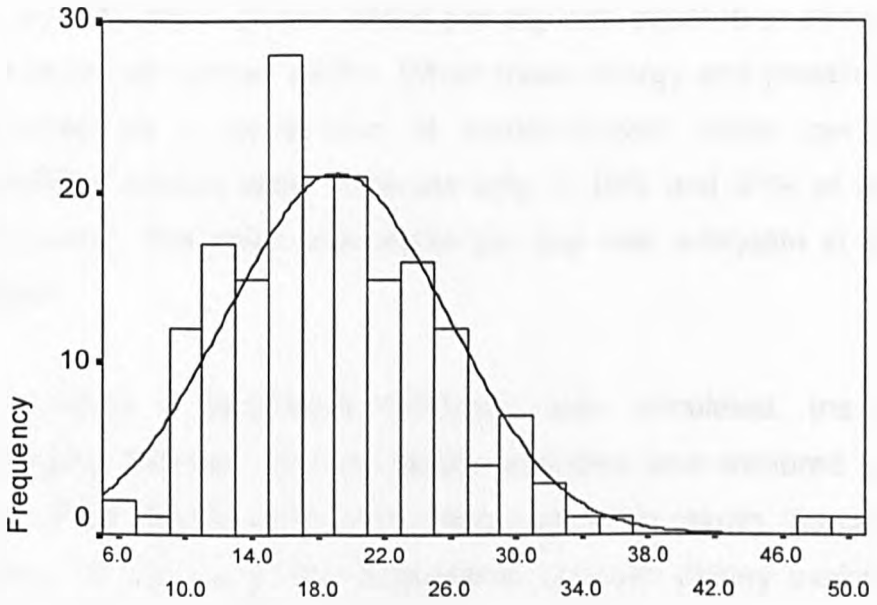
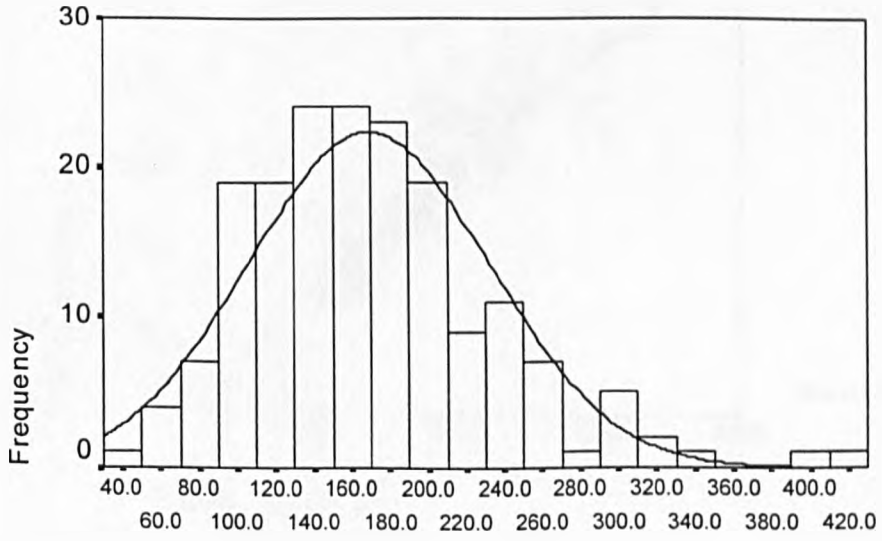


Figure 18. Histogram of iron intake expressed as a % of RDA per day



78% of the study children fell below the mean RDAs for energy intake per day. The mean protein intake per day was equal to or above the RDA in the majority of children (96%). When mean energy and protein intakes were expressed as a percentage of recommended intake per kg of body weight/day, intakes were adequate only in 16% and 61% of study children respectively. The mean iron intake per day was adequate in 87% of study children.

Before a correlation coefficient was calculated, the basic linear relationship between the two dietary variables was explored by creating a graph of the data in order to avoiding misleading results. As can be seen in Figures 19, 20 and 21 the relationship between dietary intake variables is linear. Therefore, it was appropriate to apply Pearson's correlation coefficient.

Figure 19. Scatterplot of protein intake with energy intake

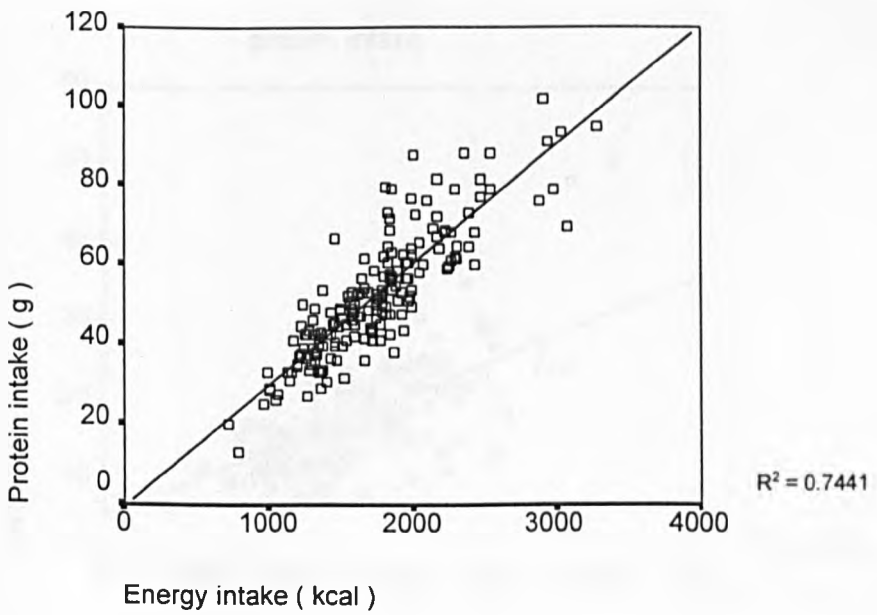


Figure 20. Scatterplot of iron intake with energy intake

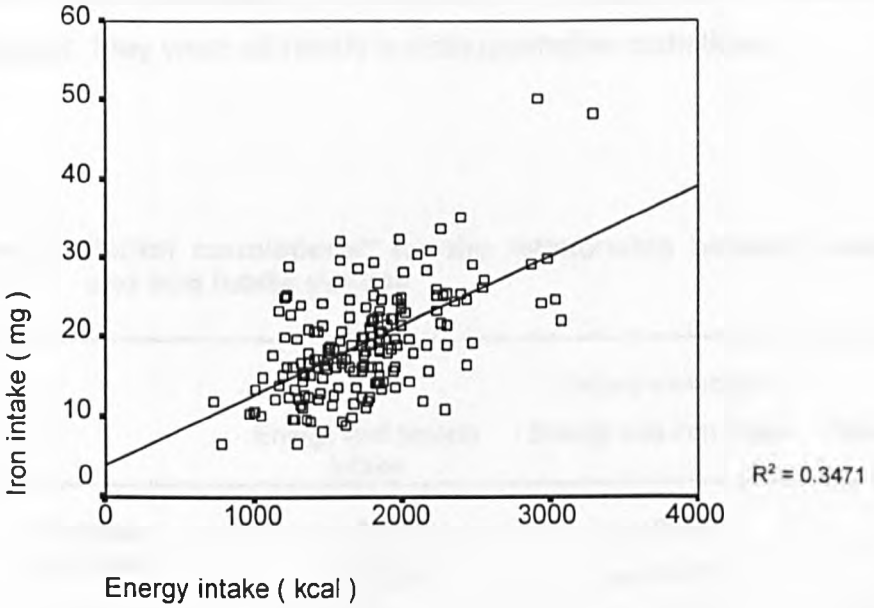
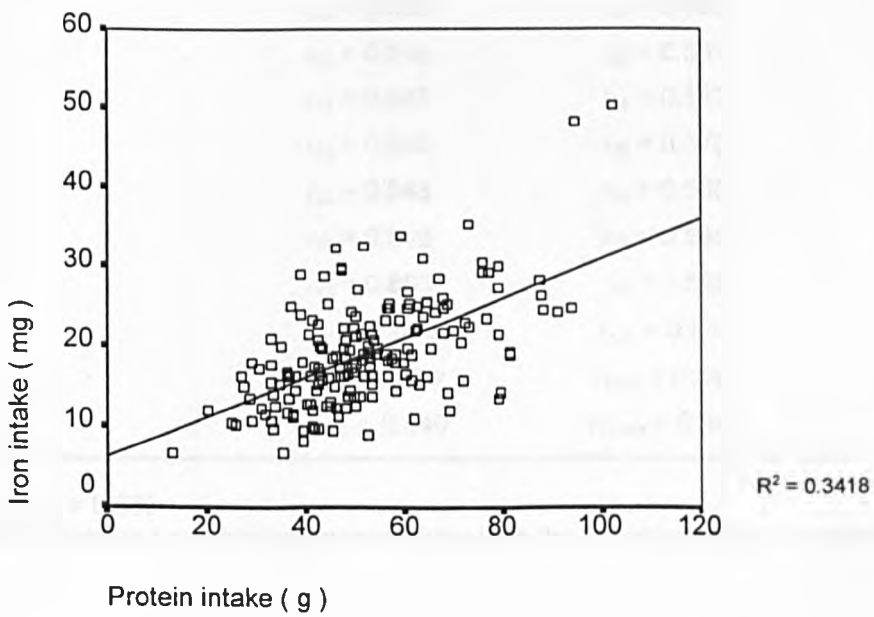


Figure 21. Scatterplot of iron intake with protein intake



Mean energy and protein intake were highly correlated (r = 0.863, p = 0.000) whereas mean energy and iron intake (r = 0.589, p = 0.000) and mean protein and iron intake (r = 0.585, p = 0.000) were modestly correlated. Moreover, they were all nearly indistinguishable statistically.

Table 21.- Partial correlations for the relationship between energy, protein and iron intake per day**

	Dietary variables:		
	Energy and protein intake	Energy and iron intake	Protein and iron intake
Variables removed:	r_1 = 0.860	r_1 = 0.583	r_1 = 0.579
	r_2 = 0.856	r_2 = 0.573	r_2 = 0.566
1.- Age	r_3 = 0.849	r_3 = 0.559	r_3 = 0.556
2.- Gender	r_4 = 0.853	r_4 = 0.576	r_4 = 0.571
	r_5 = 0.862	r_5 = 0.595	r_5 = 0.593
3.- Height	r_{12} = 0.854	r_{12} = 0.568	r_{12} = 0.562
4.- Weight	r_{13} = 0.849	r_{13} = 0.560	r_{13} = 0.557
5.- *H. pylori*	r_{14} = 0.853	r_{14} = 0.574	r_{14} = 0.570
	r_{15} = 0.859	r_{15} = 0.589	r_{15} = 0.587
	r_{23} = 0.840	r_{23} = 0.539	r_{23} = 0.534
	r_{24} = 0.845	r_{24} = 0.557	r_{24} = 0.550
	r_{25} = 0.856	r_{25} = 0.579	r_{25} = 0.575
	r_{34} = 0.848	r_{34} = 0.560	r_{34} = 0.558
	r_{35} = 0.849	r_{35} = 0.565	r_{35} = 0.565
	r_{45} = 0.853	r_{45} = 0.582	r_{45} = 0.580
	r_{123} = 0.840	r_{123} = 0.540	r_{123} = 0.534
	r_{1234} = 0.839	r_{1234} = 0.541	r_{1234} = 0.536
	r_{12345} = 0.840	r_{12345} = 0.546	r_{12345} = 0.544

** p-value = 0.000
Subscript beside r = variables removed from both the variables being correlated

The extent of the coefficients of determination (r^2) indicate that for energy and protein intake 75% of the variance of one variable is apparently explained by the other, whereas for energy and iron intake and protein and iron intake it was only 34%.

Partial correlation was used to uncover spurious correlations, moderated relationships and intervening variables. When correlation tests between two dietary variables, after controlling for the effect of one or more additional variables were carried out, it was clear that they were both unrelated to any another variable and hence the relationship was realistic (Table 21).

The Mann-Whitney U test was conducted to compare means between groups categorised by age, gender and *H. pylori* infection in order to evaluate whether any effect on dietary intake was associated with them (Table 22, 23 and 24). Since the dietary intake data was not normally distributed and the probability values for a *t* test assume that the variable is normally distributed, the Mann-Whitney U test (non-parametric) serves as an acceptable alternative, being more robust in this situation.

Table 22.- Comparison of *H. pylori* positive and negative children by energy and nutrient intake*

	H. pylori positive (n = 84)	*H. pylori* negative (n = 94)	Mann-Whitney U	Z value	p-value
Energy:					
Per day (kcal)	1711.06 ± 462.24	1771.51 ± 441.64	3547.00	– 1.168	0.243
Percentage of RDA per kg of body weight	80.57 ± 23.33	80.81 ± 20.65	3866.00	– 0.239	0.811
Percentage of RDA per day	85.55 ± 23.11	88.57 ± 22.08	3547.00	– 1.168	0.243
Protein:					
Per day (g)	50.64 ± 16.64	53.44 ± 14.85	3303.50	– 1.878	0.060
Percentage of RDA per kg of body weight	180.86 ± 59.42	190.87 ± 53.04	3650.00	– 0.868	0.385
Percentage of RDA per day	111.27 ± 38.47	113.61 ± 32.02	3303.50	– 1.878	0.060
Iron:					
Per day (mg)	19.55 ± 6.73	18.78 ± 6.68	3718.50	– 0.669	0.504
Percentage of RDA per day	172.57 ± 64.22	165.79 ± 62.94	3704.00	– 0.711	0.477

* Values are given as Mean± S.D.

There was a noticeable lack of significant difference in mean energy, protein and iron intake between *H. pylori* positive and negative children (Table 22). Also, there was a noticeable lack of significant difference in mean energy, protein and iron intake between children of both age groups, except for iron intake expressed as percentage per day (Table 23). When comparisons between groups were categorised by gender, significant differences in energy, protein and iron intake means were observed between boys and girls (Table 24). In general, mean energy and nutrient intakes were relatively higher in boys than in girls.

Table 23.- Comparison of children's energy and nutrient intake categorised by age

	9 years old (n = 95)	10 years old (n = 83)	Mann-Whitney U	Z value	p-value
Energy:					
Per day (kcal)	1691.10 ± 390.65	1802.38 ± 507.76	3353.00	– 1.719	0.086
Percentage of RDA per kg of body weight	82.50 ± 20.97	78.63 ± 22.85	3559.00	– 1.118	0.263
Percentage of RDA per day	84.55 ± 19.53	90.11 ± 25.38	3353.00	– 1.719	0.086
Protein:					
Per day (g)	50.46 ± 13.56	54.01 ±17.80	3489.50	– 1.321	0.187
Percentage of RDA per kg of body weight	114.48 ± 31.31	110.25 ± 39.12	3560.00	– 1.115	0.265
Percentage of RDA per day	180.24 ± 48.44	192.91 ± 63.58	3489.50	– 1.321	0.187
Iron:					
Per day (mg)	18.45 ± 5.96	19.93 ± 7.40	3600.00	– 0.999	0.318
Percentage of RDA per day	184.54 ± 59.68	151.20 ± 63.32	2554.50	– 4.047	0.000

* Values are given as Mean± S.D.

To examine the simple main effects and possible interaction of *H. pylori* infection with age and gender in relation to energy, protein and iron intake, the means of all groupings were compared with the use of multiple analysis of variance (Table 25). Results of the evaluation of assumptions led to transformation of the dietary variables to get them to be more like the normal distribution before multiple analysis of variance was carried out. Logarithmic transformations were used on energy, protein and iron intake per day.

Table 24.- Comparison of children's energy and nutrient intake categorised by gender

	Boys (n = 86)	Girls (n = 92)	Mann-Whitney U	Z value	p-value
Energy:					
Per day (kcal)	1835.59 ± 454.54	1656.42 ± 432.81	3127.00	– 2.413	0.016
Percentage of RDA per kg of body weight	85.49 ± 21.88	76.22 ± 21.05	3011.00	– 2.751	0.006
Percentage of RDA per day	91.77 ± 22.72	82.82 ± 21.64	3127.00	– 2.413	0.016
Protein:					
Per day (g)	55.87 ± 15.85	48.61 ±14.87	2968.50	– 2.874	0.004
Percentage of RDA per kg of body weight	121.18 ± 35.28	104.40 ± 33.17	2963.00	– 2.890	0.004
Percentage of RDA per day	199.56 ± 56.61	173.61 ± 53.12	2968.50	– 2.874	0.004
Iron:					
Per day (mg)	20.41 ± 7.20	17.95 ± 5.99	3154.50	– 2.333	0.020
Percentage of RDA per day	186.53 ± 64.10	152.60 ± 58.59	2708.00	– 3.633	0.000

* Values are given as Mean± S.D.

Significant main effects of gender (p = 0.003) on energy intake per day and interaction of age/gender/*H. pylori* infection (p = 0.037) were detected. Age, gender and *H. pylori* infection only accounted for 8.9% of the total variation in energy intake per day. There was a noticeable lack of significant effect of age, *H. pylori* infection on energy intake per day, and interaction of age/gender, age/*H. pylori* infection and gender/*H. pylori* infection.

A significant main effect of gender on protein intake per day (p = 0.001) and iron intake per day (p = 0.006) was detected, but no main effect of age and *H. pylori* nor interaction of age/gender, age/*H. pylori*, gender/*H. pylori* and age/gender/*H. pylori* (Table 25).

Table 25.- Main effects and interaction of gender, gender and *H. pylori* infection in relation to energy, protein and iron intake per day expressed as natural logarithm

Source*	ANOVA Sum of squares	Degrees of freedom	Mean Square	F value	p-value	Observed power[a]
1.- Energy intake						
*Corrected Model	0.199[b]	7	0.028	2.385	0.024	0.850
*Age	0.014	1	0.014	1.203	0.274	0.194
*Gender	0.108	1	0.108	9.081	0.003	0.850
**H. pylori*	0.011	1	0.011	0.966	0.327	0.165
*Age/gender	0.013	1	0.013	1.157	0.284	0.188
*Age/*H. pylori*	0.002	1	0.002	0.202	0.653	0.073
*Gender/*H. pylori*	0.000	1	0.000	0.011	0.916	0.051
*Age/gender/*H. pylori*	0.052	1	0.052	4.420	0.037	0.552
2.- Protein intake						
*Corrected Model	0.329[c]	7	0.047	2.743	0.010	0.903
*Age	0.008	1	0.008	0.519	0.472	0.110
*Gender	0.214	1	0.214	12.466	0.001	0.940
**H. pylori*	0.028	1	0.028	1.643	0.202	0.247
*Age/gender	0.023	1	0.023	1.349	0.247	0.211
*Age/*H. pylori*	0.007	1	0.007	0.419	0.518	0.099
*Gender/*H. pylori*	0.006	1	0.006	0.379	0.539	0.094
*Age/gender/*H. pylori*	0.052	1	0.052	3.070	0.082	0.414
3.- Iron intake						
*Corrected Model	0.285[d]	7	0.040	1.882	0.075	0.737
*Age	0.016	1	0.016	0.786	0.377	0.143
*Gender	0.170	1	0.170	7.880	0.006	0.797
**H. pylori*	0.016	1	0.016	0.747	0.389	0.138
*Age/gender	0.013	1	0.013	0.613	0.435	0.122
*Age/*H. pylori*	0.022	1	0.022	1.037	0.310	0.173
*Gender/*H. pylori*	0.049	1	0.049	2.313	0.130	0.327
*Age/gender/*H. pylori*	0.000	1	0.000	0.002	0.965	0.050

[a] Computed using $\alpha = 0.05$
[b] R squared = 0.089
[c] R squared = 0.102
[d] R squared = 0.072

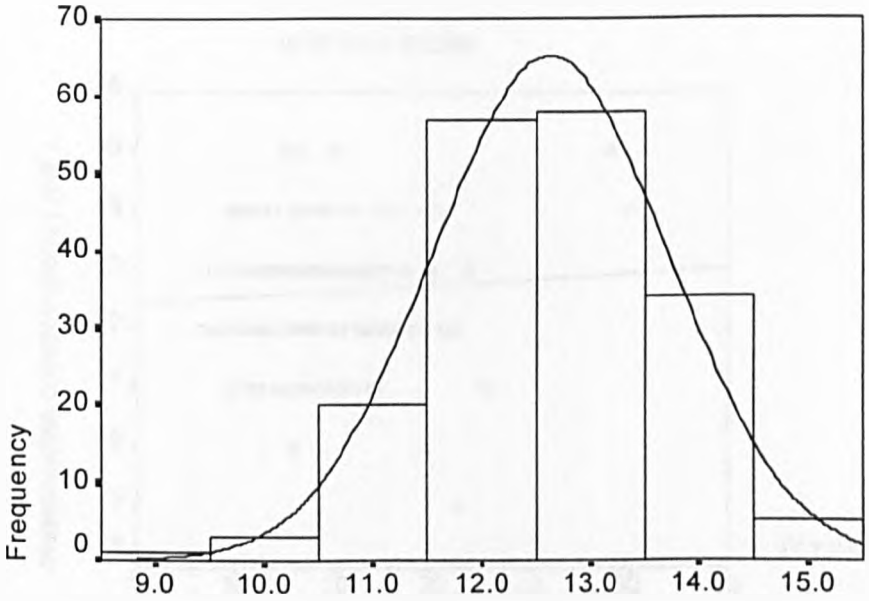
Although, the assumption for equal error of variance of energy (F = 1.081, p = 0.378), protein (F = 1.860, p = 0.079) and iron (F = 0.898, p = 0.510) intake was met, when groups were categorised by age, gender and *H. pylori* infection, there was an unequal number of cases in each group. This renders comparisons of means between groups less reliable, affecting both the results and the overall p value (apparently significant). Caution therefore must be taken in the interpretation of the significant effects and interaction detected on these dietary variables.

4.3. Iron deficiency anaemia

Since chronic recurrent infections and some intestinal parasitic infestations could be causes of anaemia and because the effects of iron deficiency consequently can result in growth deficits in childhood, this study also looked for the evaluation of these confounders at the same time.

Haemoglobin concentration (g/dl) was normally distributed (kurtosis = 0.059 and skewness = – 0.203) for the study children; with a mean of 12.6, median of 13.00, mode of 13.00 and a coefficient of variation of 0.08% (Figure 22). Before a correlation coefficient was calculated, the basic linear relationship between haemoglobin concentrations and the relationship between haemoglobin concentrations with iron, protein and energy intake per day, height, weight, height-for-age, weight-for-age and weight-for-height was explored by creating a graph of the data in order to avoid misleading results.

Figure 22. Histogram of haemoglobin concentrations



As can be seen in Figures 23 to 30 the relationship between haemoglobin concentrations with iron, protein and energy intake per day, height, weight, height-for-age, weight-for-age and weight-for-height is not linear. When the relationship between two variables is non-linear, it is appropriate to use the distribution-free correlation of Spearman's rho test for measuring associations. Haemoglobin concentration was found to be only slightly correlated with weight (r = 0.189, p = 0.011), weight-for-age (r = 0.199, p = 0.08) and weight-for-height (r = 0.230, p = 0.002). There was a noticeable lack of correlation with height, energy, protein and iron intake per day.

Figure 23. Scatterplot of haemoglobin concentrations with iron intake

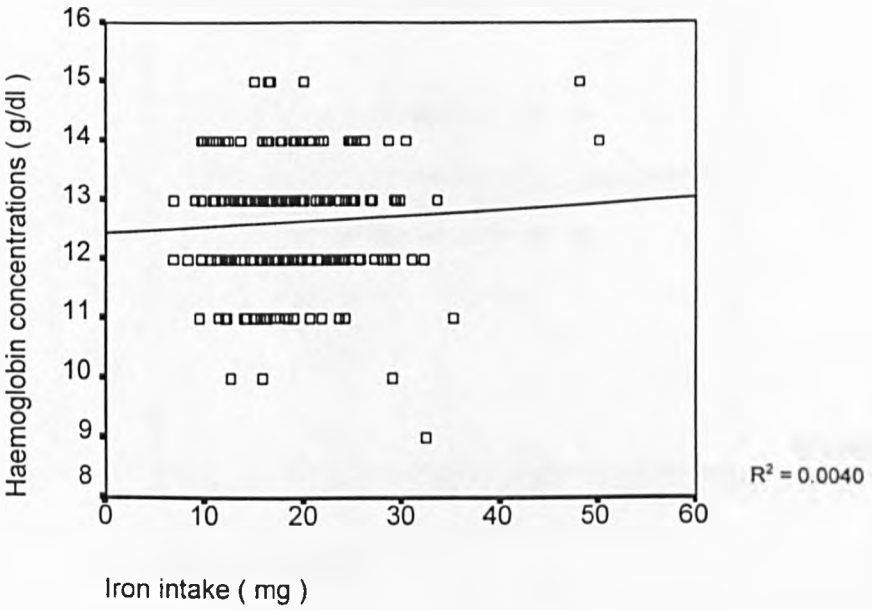


Figure 24. Scatterplot of haemoglobin concentrations with energy intake

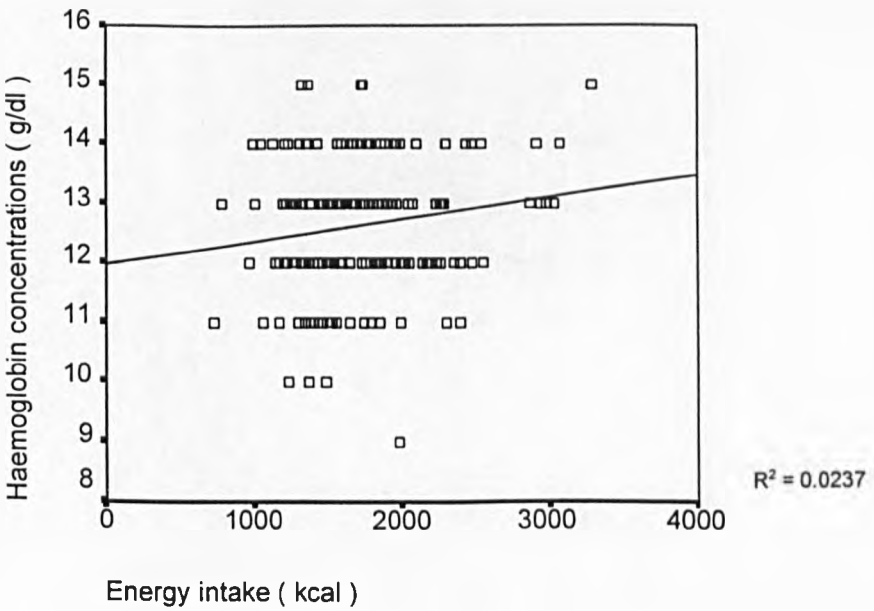


Figure 25. Scatterplot of haemoglobin concentrations with protein intake

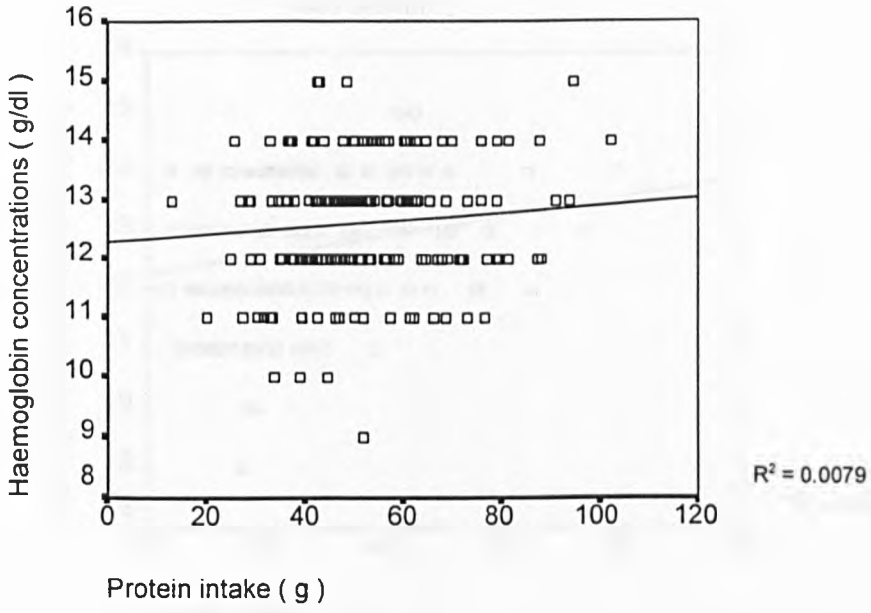


Figure 26. Scatterplot of haemoglobin concentrations with height

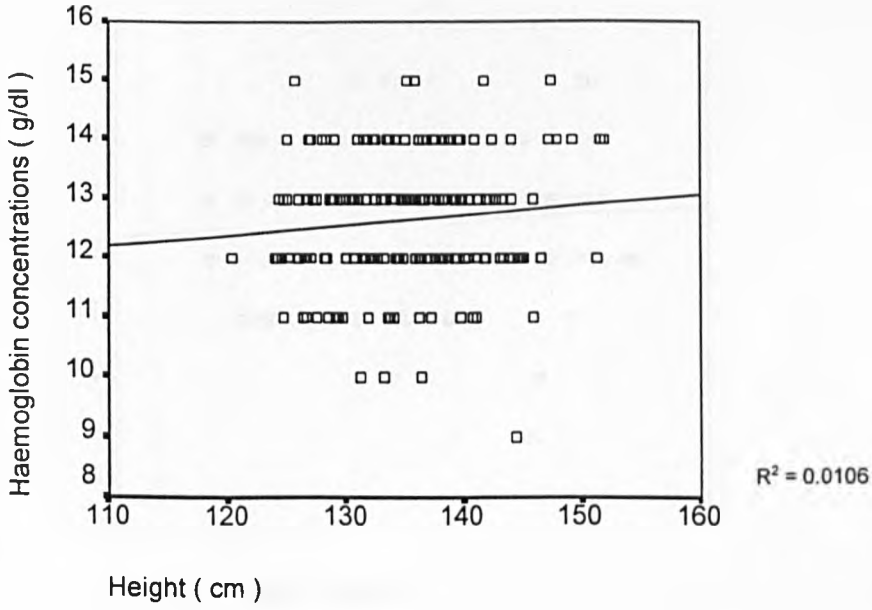


Figure 27. Scatterplot of haemoglobin concentrations with weight

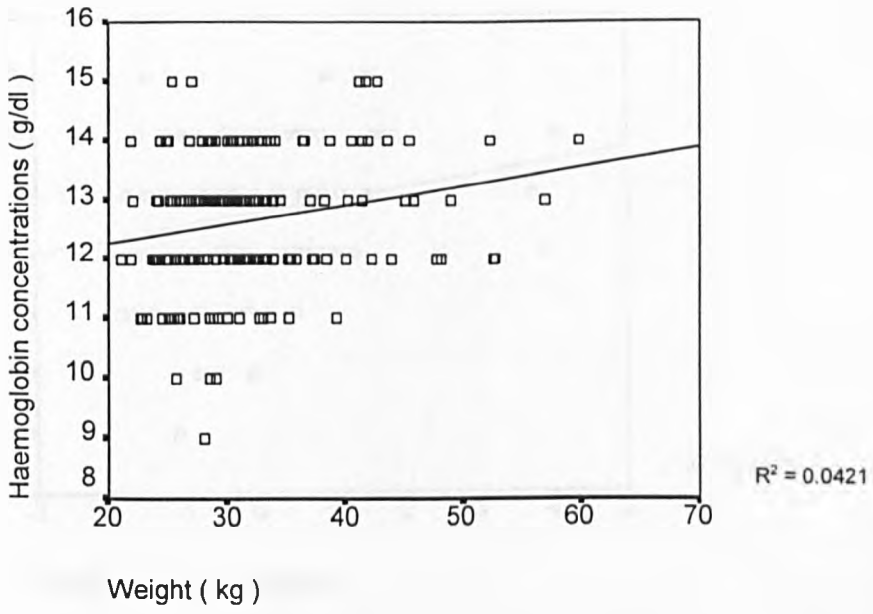


Figure 28. Scatterplot of haemoglobin concentrations with height-for-age Z-scores

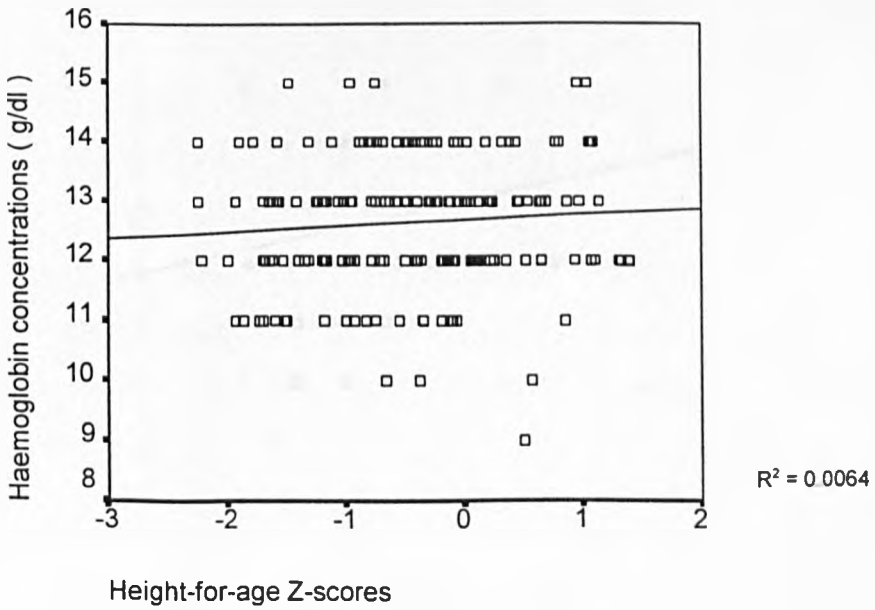


Figure 29. Scatterplot of haemoglobin concentrations with weight-for-age Z-scores

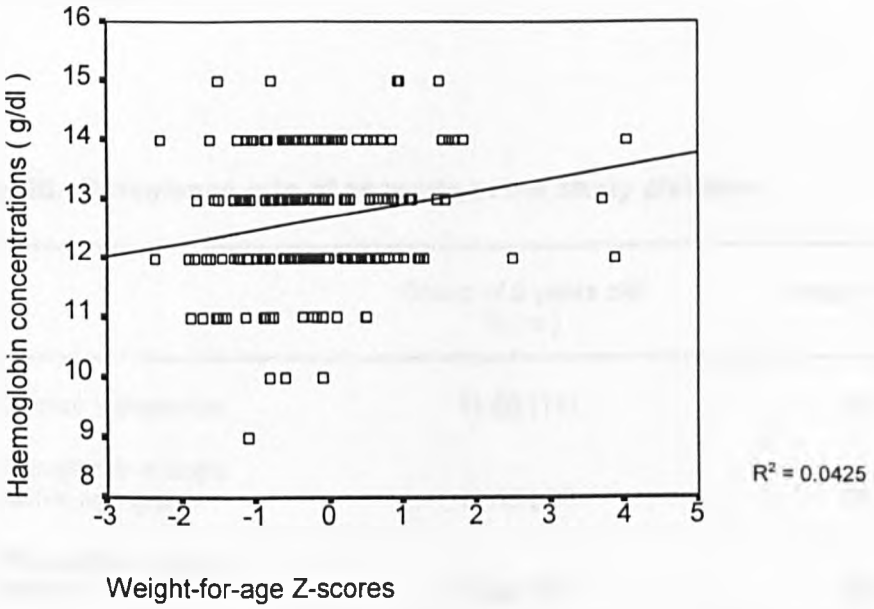
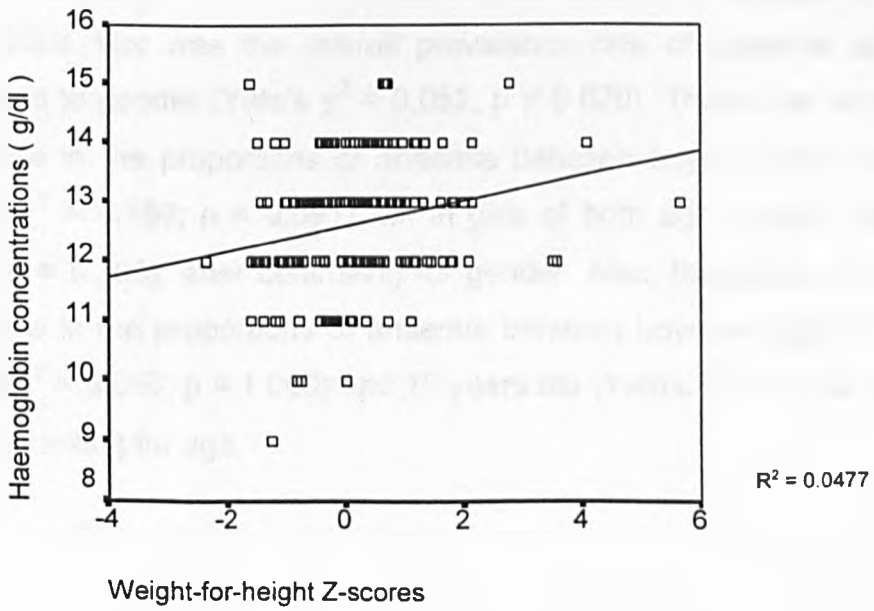


Figure 30. Scatterplot of haemoglobin concentrations with weight-for-height Z-scores



The overall prevalence rate of anaemia in the study children was only 15%. Table 26 shows the proportion of anaemic children within each age group by gender.

Table 26.- Prevalence rate of anaemia in the study children

	Group of 9 years old % (n)	Group of 10 years old % (n)
Overall prevalence	11.60 (11)	19.30 (16)
Prevalence in boys: Within age group	11.40 (5)	16.70 (7)
Prevalence in girls: Within age group	11.80 (6)	22.00 (9)

The overall prevalence rate of anaemia was found not to be significantly different between children of both age groups (Yate's $\chi^2 = 1.486$; $p = 0.223$). Nor was the overall prevalence rate of anaemia significantly correlated to gender (Yate's $\chi^2 = 0.052$; $p = 0.820$). There was no significant difference in the proportions of anaemia between boys of both age groups (Yate's $\chi^2 = 0.159$; $p = 0.691$) nor in girls of both age groups (Yate's $\chi^2 = 1.062$; $p = 0.303$), after controlling for gender. Also, there was no significant difference in the proportions of anaemia between boys and girls 9 years old (Yate's $\chi^2 = 0.000$; $p = 1.000$) and 10 years old (Yate's $\chi^2 = 0.110$; $p = 0.740$) after controlling for age.

Table 27.- Proportions of anaemic children in relation to *H. pylori* infection and the presence of intestinal parasitism

	% (n)	χ^2 value (Degrees of freedom)	p-Value
H. pylori infection			
Positive	16.7% (14)		
Negative	13.8% (13)	0.101 (1)	0.751[a]
Parasite genera			
I.- Protozoans:			
Endolimax nana			
Positive	15.0 (9)		
Negative	15.3 (18)	0.000 (1)	1.000 [a]
Entamoeba coli			
Positive	13.3 (6)		
Negative	15.8 (21)	0.025 (1)	0.876 [a]
Giardia lamblia			
Positive	6.9 (2)		
Negative	16.8 (25)	c.n.b.c.	0.259 [b]
Iodamoeba buetschlii			
Positive	50.0 (1)		
Negative	14.8 (26)	c.n.b.c.	0.281 [b]
Entamoeba histolytica			
Positive	0.0 (0)		
Negative	15.3 (27)	c.n.b.c	1.000 [b]
II.- Helminths:			
Hymenolepis nana			
Positive	13.8 (8)		
Negative	15.8 (19)	0.018	0.894 [a]
Ascaris lumbricoides			
Positive	9.1 (1)		
Negative	15.6 (26)	c.n.b.c.	1.000 [b]
Hymenolepis diminuta			
Positive	0.0 (0)		
Negative	15.3 (27)	c.n.b.c.	1.000 [b]
Trichuris trichiura			
Positive	0.0 (0)		
Negative	15.3 (27)	c.n.b.c.	1.000 [b]

[a] p-values derived from χ^2 test with Yate's continuity correction
[b] p-values derived from Fisher's exact test

The association of anaemia with positive *H. pylori* and intestinal parasites is shown in the Table 27. There was a noticeable lack of association between the presence of anaemia with *H. pylori* infection and intestinal parasites in the study children.

To evaluate whether there is any significant relation between the presence of *H. pylori* infection and haemoglobin concentrations, *H. pylori* positive and negative children were compared. No difference in the mean haemoglobin concentrations was observed between infected and non-infected children (Table 28). Since haemoglobin concentration in the study children was normally distributed and the equality of variances between groups was met satisfactorily (testing for normality), the probability of the resulting *t* value being appropriate is high. As mentioned above, haemoglobin concentration was normally distributed; however, groups of children categorised by intestinal parasites naturally divided into extremely unequal sample sized groups. It was therefore, not appropriate to examine the difference between mean haemoglobin concentrations in the infected and non-infected children groups by either the means *t* test or the Mann-Whitney U test.

Table 28.- Comparison of *H. pylori* positive and negative children by haemoglobin

	H. pylori positive (n = 84)	*H. pylori* negative (n = 94)	F value[a]	p-value[a]	*t* value[b]	p-value[b]
Haemoglobin (g/dl)	12.66 ± 1.15	12.59 ± 1.02	1.223	0.270	0.433	0.665

* Values are given as Mean± S.D.
[a] Levene's test for equality of variances
[b] Two tailed *t*-test for equality of means (equal variances assumed)

CHAPTER IV

DISCUSSION

This chapter is subdivided into three sections. The first section discusses the main results on prevalence of and potential risk factors for *H. pylori* infection within the study population setting and compares it with previous findings. The second section presents an examination of *H. pylori* infection in study children and its possible effects on growth, while accounting for factors that may modify the effects of *H. pylori* infection. The third section highlights the strengths and research limitations of the findings in relation to methods used in this study.

1. Prevalence of and Potential Risk Factors for *H. pylori* Infection

This study showed a high overall prevalence rate of *H. pylori* infection (47.1%) among the study children aged 9-10 years attending school in Hermosillo, Sonora, Mexico. The prevalence rate is very similar to that reported in previous studies carried out in Mexico (Nurko et al, 1993; Ramírez-Mayans et al, 1997; Torres et al, 1998). This prevalence rate is higher than that of developed countries where infection is relatively rare among children (Graham et al, 1991; Matysiak-Budnik and Mégraud, 1994; Patel et al, 1994). The prevalence rate of *H. pylori* infection among study children, however, was lower than those reported in some other poor populations from developing countries, which may reflect more acceptable living conditions in this study population (Wright et al, 1987; Miller at al, 1988; Katelaris et al, 1992; Clemens et al, 1996).

Nevertheless, this finding is consistent with other studies in children in that a higher prevalence rate of infection is associated with lower socio-economic status (Fiedoreck et al, 1991; Hopkins et al, 1993; Oliveira et al, 1994). Although the inverse relationship between *H. pylori* infection and lower socio-economic status has been widely reported, it remains largely unaccounted for.

This study is the only cross-sectional epidemiological study of the prevalence of *H. pylori* infection in children of Mexico, using the ^{13}C-urea breath test, known to be both highly sensitive and specific. In Mexico, two studies on children (Nurko et al, 1993; Ramírez-Mayans et al, 1997) and a retrospective serologic population-based study (Torres et al, 1998) have provided data relating to the rate of prevalence of *H. pylori* in children. Although these studies gave some preliminary data, they are unlikely to reflect the true prevalence of *H. pylori* infection in Mexican children. *H. pylori* infection status in many studies has been determined by using less accurate diagnostic tests, such as serological or salivary IgG, and obtained from highly selected samples; hence, a real comparison between estimated prevalence rates is inevitably limited

The prevalence rate of *H. pylori* infection increases with age, but the association with gender in children has not been uniformly reported among and within varied geographical regions. Here, overall infection prevalence was not found to be related to gender. However, the observed differences in the prevalence rate of *H. pylori* infection, after controlling for gender/age might merely reflect differences in levels of risk factors and may be responsible for the observed pattern.

This difference in prevalence could in theory be due to differences in genetic susceptibility to environmental determinants between children, but might also result from other factors within and between families such as behaviour involving close contact that may be dissimilar.

H. pylori infection was not significantly correlated with the child's birthplace, even after controlling for age and gender. In the bivariate analysis, both rural-born mothers and fathers were strongly and significantly correlated with increased *H. pylori* infection prevalence in study children even after controlling for gender and age. A previous large study in China, however, suggests a lower prevalence rate of infection in rural areas than in urban, but this finding is difficult to interpret due to possible misclassification of *H. pylori* status by serodiagnosis (Mitchell et al, 1992). On the other hand, one population-based study in Mexico found no difference in *H. pylori* infection between urban and rural communities (Torres et al, 1998). The inconsistency from this study may be explained by the fact that only the current residence was taken into account, lacking the precision of data on the true rural/urban birth origin of the study population, as well as possible misclassification of *H. pylori* status by serodiagnosis.

The strong effect of a rural-born father on an increased *H. pylori* infection prevalence in the study children, observed by the logistic regression analysis, lacks evidence in previous studies. This observation is difficult to interpret without data on father's age at migration and duration of time spent in the new environment relevant to the child. It is not clear, whether or not these differences could have existed between children before the father's migration, or whether children became infected afterwards.

It is possible that families with migrant fathers could preserve certain environmental determinants of the current risks that are strongly correlated with father's rural origin or that after coming to a peri-urban setting the child became infected due to an increased risk due to changes in living conditions. Therefore, it is difficult to declare that people of rural origin have a particular susceptibility to *H. pylori* infection. A more detailed analysis of child/parent's rural origin might shed more light on this association.

The findings of this study indicate that household overcrowding is a less important risk factor for acquiring the *H. pylori* infection than number of children, sharing a bed in childhood and type of main water supply (one tap in the yard), confirming results of other previous studies (Klein et al, 1991; Mendall et al, 1992; Whitaker et al, 1993; Webb et al, 1994; Goodman et al, 1996). Therefore, household overcrowding may be only an intermediate variable or marker for other factors, which indirectly may affect the mode of transmission of *H. pylori* infection. Since in this study, data on number siblings, the sharing of beds by the study child and type of main water supply were observed directly, the possibility of biased associations between *H. pylori* infection and these factors due to misclassification should be minimal.

Indeed, the findings of this study are consistent with previous studies in that number of children and sharing a bed in childhood are the strongest predictors of *H. pylori* infection. In addition *Hymenolepis nana*, a person-to-person transmitted parasitic infection, was also observed in study children with a high overall prevalence rate. This favours the hypothesis that the most likely mode of transmission of *H. pylori* infection may be spread by direct person-to-person contact, especially among children in developing countries

Other studies have also found that the prevalence rate of *H. pylori* infection is raised in the families of index positive children and among residents of closed communities such as orphanages and psychiatric institutions (Drumm et al, 1990; Pérez-Pérez et al, 1990; Vicent et al, 1994; Ramírez-Mayans et al, 1997). In developed countries, improved living conditions, trends towards smaller family size and much lower density of living may currently account for lower prevalence rates of *H. pylori* infection in children. Since *H. pylori* infection is acquired primarily in childhood in developing countries, further studies of transmission in these children, including their families, may be more revealing.

In the bivariate analysis, this study found that the overall prevalence of *H. pylori* infection is associated with the type of main water supply (one tap in the yard) and significantly so after the test of direct logistic regression model. This finding is consistent with one previous study from Peru, in that children whose homes had access solely to one external tap were more likely to be infected than those in homes with internal taps (Klein et al, 1991). Furthermore, a recent study in Colombian children in a rural community found that *H. pylori* infection was commoner in children who reported drinking from local streams at some time (Goodman et al, 1996). Although most Colombian families reported boiling their drinking water before use (initiated during the preceding 3 years), no clear associations were observed between current or past boiling water practices and *H. pylori* infection prevalence. One possible explanation of this finding is that, once acquired, *H. pylori* infection is likely to be for life, but also that spontaneous clearance of infection without treatment may also occur.

Nevertheless, the large study from China found no significantly associated source of drinking water with *H. pylori* infection; however, this may be explained by the fact that most people boiled their drinking water (Mitchell et al, 1992). In addition, Mendall et al (1992), have reported an association between lack of a fixed hot water supply in the childhood home and seropositivity in adult life. In contrast, a study in Korean children found no significant association between *H. pylori* infection and source of water supply (Malaty et al, 1996). However, this data from Korea must be regarded with caution, as the sources of drinking water were only roughly classified and additional information related to boiling water practice or homes with an external tap was not noted. Therefore, it is possible that the significant negative association may reflect confounding by other inadequately controlled variables.

These previous findings suggest that this study's results may in part be explained by the generalised practice in the Hermosillo population of drinking unboiled tap water. It is possible that the habitual consumption of inadequately treated water and/or the presence of other environmental factors may influence the mode of transmission of *H. pylori* infection. In agreement with that, a high overall prevalence rate of waterborne enteric parasitic infections such as *Endolimax nana* and *Entamoeba coli* was observed in study children. Although this finding suggests that contaminated water could be an important source and vehicle for transmission of *H. pylori* infection, statistical evidence has not yet been provided by other studies. Still, rigorous cultural practices regarding drinking water hygiene such as boiling water could be a protecting factor in the acquisition of *H. pylori* infection. This question remains to be evaluated in future studies.

In this study the prevalence rate of *H. pylori* infection was not significantly correlated with the presence of animals at home, neither with direct contact with indoor/outdoor animals by study children, even after controlling for age and gender. This finding is concordant with a recent study in children from Germany in that *H. pylori* infection was not associated with the presence of pets (rabbits, cats and dogs) in the household or with contact with pets outside the home (Bode et al, 1998). In addition, *H. pylori* infection in children was also negatively associated with pet ownership in a study from Arkansas, USA (Fiedorek et al, 1991).

On the other hand, analogies with *H. pylori* infection caused in experimental animals (to germ-free dogs) suggest that the infection might be spread from an animal reservoir (Radin et al, 1990). The study from rural Colombia found that children who had contact with sheep had increased prevalence of *H. pylori* infection (Goodman et al, 1996). This finding supports the strong probability that livestock animals might be a reservoir for human infection and ruralism an important risk factor; which is consistent with the strong effect of rural-born father on the increased prevalence of *H. pylori* infection observed in this study. However, the relationship of exposure to indoor/outdoor animals and *H. pylori* infection is still uncertain. Therefore, well-designed epidemiologic studies are needed before animal-to-man spread can be ruled out.

Despite the low socio-economic level of the population studied, they were not living under conditions of extreme poverty, inasmuch as all families had some source of income, access to food and their own house. However, the physical environment is peri-urban and poor sanitation is still common among most families.

In summary, the results of this study indicate that low socio-economic level *per se* is a less important risk factor, which may be only an intermediate variable or marker for the other micro-environmental factors that may influence the mode of transmission. As demonstrated for intestinal parasitic infections, the higher prevalence rate of *H. pylori* infection may be particularly related to poor housing, household overcrowding, lack of sanitation and ruralism. Hence, the overall prevalence rate of *H. pylori* infection could be used as an independent indicator of social underdevelopment and inequalities.

In this study population, family socio-demographic characteristics and living conditions were observed to be very similar, thus, allowing in depth examination of particular risk factors that may be masked in more heterogeneous population-based studies.

2. *H. pylori* Infection and its Possible Effect on Growth

The growth of study children was observed to be similar to the reference values of the National Center for Health Statistics (NCHS). No differences in the mean weight-for-age and weight-for-height Z-scores were observed between *H. pylori* positive and negative children. The results also suggest some height-for-age Z-score differences between infected and non infected children. Although there are relatively few studies of this type for comparison, the study's results are consistent with some previous cross-sectional studies in populations from developing countries and developed countries as well (Sullivan et al, 1990; Raymond et al, 1994; Patel et al, 1994; Weaver et al, 1994; Perri et al, 1997).

It is known that physical growth is under neuro-endocrine, environmental and genetic control, although the precise effects of genetic factors on growth are still only partly understood. It has been estimated that genetic factors contribute by about 60% to difference in adult stature (Mueller WH, 1986). Thus, genetic factors could be one possible explanation for this height-for-age Z-scores difference found in this study, but this is unlikely in this population, which has a low overall prevalence rate of malnutrition of 4.4% reported for the children of this population (SSA, 1997). Their growth pattern is also shown to be very similar to that of the well-off populations from developed countries (SEP/DIF, 1993; Jiménez-Guerra and Roman-Pérez, 1994).

It is generally accepted that adult size and shape is the result of a complex interaction between genetic and environmental factors. During childhood, however, environmental factors such as diet, disease and psychosocial stress are the major determinants of differences between populations.

It has been suggested that adverse family and social environment, as such, can slow down physical growth, but the complex mechanisms by which this happens are still largely unknown (Skuse et al, 1994). Although it was not possible at the time to measure variables related to socio-emotional deprivation within the study children families, these circumstances seem to be relatively uncommon in this study population, even living in a socio-economically disadvantaged environment.

Indeed, of critical importance in *H. pylori* positive children is the presence of co-existing enteric parasitic infections and their simultaneous effects on growth.

It is well documented that infectious diseases depress the appetite, inhibit the absorption of nutrients, alter the body's metabolism and consume energy in fighting off infection; consequently, fewer nutrients are available for maintenance and growth in the child (Martorell et al, 1975; Stephenson et al, 1989; Lunn et al, 1991;). This in turn compromises the body's defences and increases the vulnerability to illness; as a result, nutrient pools are further depleted, initiating a vicious circle that leads to poor growth and continuing delay in growth and development. To date, no previous studies have examined whether data pointed to associations between *H. pylori* infection with any of the enteric parasitic infections of public health importance, as well as whether the association was interactive or synergistic in relation to growth.

Here, the small height-for-age Z-score differences seen in the study children do not appear to result from negative effects of enteric parasites. However, *Hymenolepis nana* was found to be positively and highly associated with *H. pylori* infection. This finding lacks evidence in the literature on *H. pylori* infection in children and its effects on growth. Furthermore, it has not been proved that *Hymenolepis nana* affects growth. However, the evidence that *Hymenolepis nana* can remain attached to and damage the surface of intestinal mucosal (causing inflammation and haemorrhage) suggest that some nutritional implications may be present. Since the main effects and possible interaction between both *Hymenolepis nana* and *H. pylori* with gender could not be examined in this study, the possibility of either synergistic or antagonistic effects at the clinical or sub-clinical level must hence be considered in future studies.

Although the evidence concerning the role of energy, protein or iron intake on improved growth in children is conflicting (Gopalan et al, 1973; Lampl et al, 1978; Martorell and Klein, 1980; Chwang et al, 1988), it is well known today that inadequate dietary intake influences linear growth as well as other nutrition outcomes. However, no previous studies have examined whether the nutrient intake of the diet is associated with delayed growth in children infected with *H. pylori*. The lack of this type of data is particularly critical for comparison with findings of this study.

In this study, the differences in energy, protein and iron seen between boys and girls do not appear to result from negative effects of *H. pylori* infection or due to age. The findings suggest that these differences may be due to gender. This may largely be explained by differences in body size, physical activity and cost of growth, which were not measured in this study. Since the study also measured dietary intake in a group of children with *H. pylori* infection and enteric parasitic infections, the normal intakes of some children could also be altered by the demand of these infections. A further possible explanation of the observed differences is that the need to record dietary intake could result in modification of usual eating patterns.

In addition, the "flat slope syndrome" in twenty-four-hour recalls has been reported, which produces a downward bias in the number of subjects with uncommonly low/uncommonly high intakes and may vary with the age and gender (Gersovitz et al, 1978; Burema et al, 1988).

On the other hand, the 24-hour dietary recall could have provided useful information on dietary intake from other point of view, not necessarily correlated with *H. pylori* infection.

The dietary information obtained from the study children recorded as average nutrient intakes over 3 days may offer important and valid data for this population. The limitation of this method and the possible lack of precision in dietary data at individual level in this epidemiologic study must be counterbalanced by the large sample size as well as approaching of analysis at population level.

The study's results suggest that neither protein nor iron but only energy intake could have played a small part in determining the pattern of growth faltering in study children. However, there have been conflicting reports on the effect of energy deficiency on growth; for example, the finding from the Nutrition Collaborative Research Support Program (CRSP) that Mexican and Egyptian pre-school children showed no association between larger size and faster growth with a higher energy intake (Beaton et al, 1992). Moreover, linear growth faltering was common in Egyptian, Kenyan and Mexican pre-school children even though their protein and essential amino acid intakes were adequate. In contrast, inadequate vitamins or minerals intakes were observed in Mexico, Egypt and Kenya. This data supports the strong probability that growth retardation may be determined by simultaneous multiple micronutrient deficiencies, making it quite difficult to find an association between the intake of an individual nutrient and delayed growth in free-living children.

It is generally accepted that adequate bodily iron stores are essential for adequate growth during infancy and childhood. A number of studies have reported the benefits of iron supplementation for linear growth and weight-gain of anaemic children (Chwang et al, 1988; Latham et al, 1990). The evidence is conflicting, however, because the response in children who are not anaemic is less predictable.

Although iron-deficiency anaemia can be caused by or aggravated by malabsorption due to parasites, it has not yet been reported for *H. pylori* infection. In this study, the presence of iron-deficiency anaemia was low and was not associated with *H. pylori* infection and intestinal parasites nor was difference observed in the mean haemoglobin concentration between *H. pylori* positive and negative children. Therefore, the observed small height-for-age Z-score differences seen in the study children may not be due to iron deficiency. Since the possible effect of *H. pylori* infection and/or intestinal parasites on iron status may be dependent on several factors such as intensity of the infection, previous dietary iron intakes and gender/age, it is not extraordinary that any relationship could be not so clear in this study. Indeed, also consideration is needed in the interpretation of this finding because of the relative insensitivity of haemoglobin as an index of iron deficiency.

Even though unmeasured or poorly measured confounding remains a possible explanation for the findings in this study; if due attention is paid to critical issues, it is possible to credibly infer a small effect of *H. pylori* infection on growth. On the other hand, it should be mentioned that statistically significant results are not in themselves meaningful unless accompanied by clinically significant results or have relevance in public health.

Although no definitive conclusions are warranted, the data presented suggest the need for caution in the interpretation of previous findings from published studies on the negative effects of *H. pylori* infection on linear growth. Because of methodological weaknesses in previous studies on *H. pylori* infection and the established effects of individual/simultaneous nutrient deficiencies and enteric parasitic infections on physical growth, it is plausible that studies attributing the effects of *H. pylori* infection on linear growth may be not entirely true.

No study on *H. pylori* infection has been able to take into account the effect of potential confounding by factors such as dietary intake and/or consequences from other co-existing enteric parasitic infections causative of impaired nutrient status by different means. Such effects could have been produced by an inadequate intake of energy and/or protein, rather than infection by itself. Neither the role of other simultaneous multiple micronutrients deficiencies nor the effects of physical activity on somatic growth can be discounted. Such previous studies may also be misleading if they did not consider this potential confounding factor, because it is difficult to separate the true effect of *H. pylori* infection from that of dietary intake and/or physical activity. The published data do not allow for a retrospective assessment of possible additive or interactive effects from these other co-existing conditions.

Most of the studies that approached the relation between delayed growth and *H. pylori* infection have examined the effect of socio-demographic confounders, with less emphasis on interactive biological variables. It would be important to include confounding variables that could influence the child's growth such as size at birth (most specifically length), genetic factors, and parental factors (primarily related to stature and degree of fatness/thinness) in future studies. If possible, further information on appetite, physical activity, bacterial overgrowth, malabsorption, and quality of the family environment (psychological and socio-emotional aspects) should be collected.

Present criteria to judge the immediate negative effects or long-term consequences of *H. pylori* infection on linear growth should not exclusively rely on somatic measurements but also on growth hormone and insulin-like growth factor-I levels in plasma and growth hormone in urine as suitable indices of the hormonal regulation of growth.

On the other hand, *H. pylori* secretes several factors including potent neutrophil chemotactis factors and substances capable of activating peripheral blood monocytes to produce the cytokines Interleukin 1β, tumour necrosis factor α, and Interleukin-6, and induce secretion of mucosal Interleukin-8. The intestinal inflammatory response, Interleukin-1β and tumour necrosis factor α appear to be responsible for causing delayed gastric emptying, anorexia, diarrhoea and weight loss (Plata-Salaman, et al 1988). Thus, consideration should also be given to measurements of cytokine response (particularly tumour necrosis factor-α and IL-1β) plus plasma transferrin as biochemical indices of metabolic effects of infection.

It must be emphasised that a strong relationship between *H. pylori* infection and linear growth retardation arising from a cross-sectional study is not a straightforward way of indicating that *H. pylori* infection causes linear growth retardation. Previous studies are cross-sectional, therefore, it is impossible to determine whether *H. pylori* infection leads to failure in reaching linear growth potential in children, whether linear growth retardation predisposes children to be infected by *H. pylori*, or whether both phenomena to some extent occurred simultaneously. It could well be that failure to grow might be a result of interactive/additive effects of other factors such as co-existing parasitic infections and/or inadequate dietary intake together with *H. pylori* infection. The possibility of confounding and the effect of modification make the interpretation of epidemiologic studies more difficult.

On the other hand, the borderline significant small effect of *H. pylori* infection on height-for-age Z-scores found in this study conflicts with results from other studies. Conflicting evidence comes from three cross-sectional studies carried out in developing countries populations.

Oliveira et al (1994), observed no significant difference in the seroprevalence of *H. pylori* infection related to eutrophic and malnourished 1 month to 18 year-old Brazilian children. This result might be explained in part by possible misclassification of *H. pylori* status by serodiagnosis and misinterpretation of physical growth status due to use of non-anthropometric indices such as height-for-age and weight-for-age.

Mahalanabis et al (1996), reported that *H. pylori* infection (detected with ^{13}C-urea breath test) was not associated with poor nutritional state as measured by weight-for-age, in 1 to 99 month-old Bangladeshi children. Although in this study misclassification of *H. pylori* status may be present minimally, one intriguing aspect is that growth was evaluated using only weight-for-age expressed as a percentage of reference value instead of by Z-scores. Moreover, weight-for-age is influenced by height (height-for-age) and weight (weight-for-height) making its interpretation complex.

Clemens et al (1996), found that seropositivity for *H. pylori* infection was not associated with poorer weight-for-age, weight-for-height and height-for-age in 2 to 9 year-old Bangladeshi children. Although in this study physical growth status was assessed and interpreted properly, misclassification of *H. pylori* status by serodiagnosis cannot be discounted. In addition, this study was carried out in an area with high prevalence of malnutrition, which could have generated conflicting results. The discrepant results of previous studies could in theory be due to selection bias, misclassification of *H. pylori* status, misapplication and misinterpretation of anthropometric measurements as well as confounding factors. Therefore, caution is needed in accepting encouraging results of previous studies because of the lack of methodological rigour in their design and data analysis.

In summary, the reasons for any given impairment of child's growth are extremely complex. It is very difficult to isolate the effects of *H. pylori* infection. The data of this cross-sectional study have emphasised the difficulties of analysing the relationship between the *H. pylori* infection and delayed growth in children to draw causative rather than simply associative conclusions.

The small effect of *H. pylori* infection on growth observed in this study needs further confirmation as well as further consideration of discrepant results before arriving at conclusions regarding the true statistical significance and clinical/public health meaning of this finding. Therefore, data from well designed study "randomised, controlled community trials of treatment of *H. pylori* infection" that could produce changes in linear growth velocity and/or prospective large-scale cohort studies that adequately consider confounding are needed before no effect of infection on growth in children can be ruled out. If necessary, statistical analyses should consider potential confounding factors and the presentation of results should be sufficiently detailed to admit assessment and criticism.

3. Research Limitations

The limitations in this study must be acknowledged. First, the design of this study was cross-sectional. Secondly, in this study there is likely to be one potential source of measurement error. Some degree of misclassification of iron status could result, because of the relative insensitivity of haemoglobin as an index of iron status. These could in turn contribute to underestimate any possible association with *H. pylori* infection. Thus, association could result between iron-deficiency anaemia and *H. pylori* infection and/or enteric parasitic infections.

Third, a further limitation of this study is the fact that information on dietary intake was ascertained by the 24-hr recall method without verification by biomarkers of dietary exposure. As far as is known, however, the major tools of dietary exposure assessment currently in use are subjective, biased measures of the total exposure. Weighed records could have provided a greater degree of accuracy in estimating energy and nutrient intakes, but they could have tended to alter normal intakes because the method operates prospectively and the subjects record all their foods eaten. In addition, weighed recording is a time-consuming and costly method, which represent an obstacle for use in large epidemiologic studies and particularly in children. Fourth, there are the potential limitations of the analysis of this cross-sectional study, which may affect inferences, derived from the results.

However, the strengths of the study must also be highlighted. The formulae used above to get the sample size for this study helped to ensure a significant outcome. Children of the same family were not enrolled in order to prevent bias by known familial clustering of *H. pylori* infection. In addition, stratified sampling ensured a greater degree of representativeness and decreased the probability of sampling error.

Misclassification of *H. pylori* status was unlikely, since the ^{13}C-urea breath test is known to be both sensitive (90-98%) and specific (95%) for *H. pylori* infection. Although causes of specific gastric symptoms, such as alcohol, smoking and non-steroidal anti-inflammatory drug use are generally confounding variables in adult subjects, their effects were unlikely to be present in this study of 9-10 year old children. In addition, biased assessment was unlikely, because at the time of examination the diagnosis was totally unknown and all subjects received the same scrutiny. This is also the case for enteric parasitic infections.

Since the cases in the study sample truly represented the *H. pylori* infection of the whole study population, any contrast between cases and the healthy subjects could not be the result of selection bias. In a sample as large as that of this study, there is less chance that the correlation could be simply the product of sampling error.

Bias in assessment of height and weight was unlikely, since at the time measurement the persons doing it were unaware of the diagnosis of each child. Recordings taken were accurate, because instruments were calibrated regularly. Since children aged over 10 years were excluded, biased results due to anthropometric changes associated with puberty are very unlikely. The possibility of biased associations in this study did not arise from the selection of subjects and/or a low participation rate.

CHAPTER V

CONCLUSIONS

The family socio-demographic characteristics and living conditions of the population studied were observed to be very similar. Despite the low socio-economic level, they did not live under conditions of extreme poverty, inasmuch as all families had some source of income, access to food and their own house. However, their physical environment is peri-urban and poor sanitation is still common among most households.

H. pylori infection in children aged 9 and 10 years attending school in the poorest sectors of the city of Hermosillo, Sonora, Mexico is common, with a high prevalence rate of 47.1%. *H. pylori* infected children were asymptomatic carriers.

In the bivariate analysis, the overall prevalence rate of *H. pylori* infection was not found to be related to either gender nor to the two age groups. It was not related to child's birthplace even after controlling for age and gender. However, statistically significant differences in the proportions of *H. pylori* positive boys between the two age groups and between boys and girls infected aged 9 years were observed, after controlling for gender and age. Also, there was a strong and significant correlation between *H. pylori* infection prevalence rate and rural-born parents.

In the bivariate analysis, household overcrowding, the number of siblings, the sharing of beds by the study children and siblings, sleeping in the kitchen and type of main water supply (one tap in the yard) showed a strong statistical association with *H. pylori* infection prevalence.

However, there was a noticeable lack of statistical association with the sharing of a bedroom by the study child, the sewerage system and the type of excreta disposal. Nor was the prevalence rate of *H. pylori* infection significantly correlated with the presence of animals at home, nor with direct contact with indoor/outdoor animals for study children, even after controlling for age and gender.

Rural-born father, number of siblings (≥ 3 per family), the type of main water supply (one tap in the yard) and the sharing of bed by the study child were observed as potential risk factors for acquiring the infection. The test of this model of *H. pylori* status on these four explanatory variables had an overall correct prediction rate of 68.3%. The apparent associations between *H. pylori* infection with mother's birth place, household overcrowding, sleeping in the kitchen and presence of hens in the homes are spurious because they did not contribute to the prediction of infection status.

In this study, a borderline significant difference in the mean height-for-age Z-scores between *H. pylori* positive and negative children was observed. The extent of the effect for this difference is 0.27, signifying a small effect of *H. pylori* infection on height-for-age Z-scores. On the contrary, no difference in the mean weight-for-age and weight-for-height Z-scores were observed between infected and non infected children; hence *H. pylori* had not have any effect on these indices.

In the multiple analysis of variance, a borderline significant main effect of *H. pylori* infection on height-for-age Z-score was detected but no main effect of gender nor interaction of *H. pylori* infection by gender.

However, *H. pylori* infection and gender only accounted for 3.6% of total variation in height-for-age Z-scores. In addition, there was a noticeable lack of significant effect of *H. pylori* infection on weight-for-age and weight-for-height Z-scores as well as of gender and interaction of *H. pylori* infection by gender.

On the other hand, 22% of study children were neither infected with enteric parasites nor with *H. pylori*. However, about 40% of study children were found to be infected and harbour more than 1 parasitic infection as well as being infected by *H. pylori* infection at one time. *H. pylori* infection was found to be positively highly associated with *Hymenolepis nana*. There was a noticeable lack of association with other parasitic infections. No difference in the mean height-for-age, weight-for-age and weight-for-height Z-scores were observed between infected and non-infected children with each enteric parasitic infection. It was not appropriate to examine the main effects and possible interaction of *H. pylori* infection, *Hymenolepis nana* and gender in relation to height-for-age Z-scores.

There was no difference in mean energy, protein and iron intake between *H. pylori* positive and negative children and between both age groups, except for intakes expressed as percentage per day. However, significant differences in the mean energy, protein and iron intakes were observed between boys and girls. In general, boys had energy and nutrient intakes relatively higher than girls. Significant main effect of gender on energy intake per day and interaction of age/gender/*H. pylori* infection were detected. Still, no significant effect of age and *H. pylori* infection on energy intake per day, neither interaction of age/gender, age/*H. pylori* infection and gender/*H. pylori* infection was found.

A significant main effect of gender on protein and iron intake per day was detected but no main effects of age and *H. pylori* infection nor interaction of age/gender, age/*H. pylori* infection, gender/*H. pylori* infection and age/gender/*H. pylori* infection. Nonetheless, caution is needed in accepting these stimulating results on main effects and interactions because of the lack of equal number of cases in each group of energy, protein and iron intake categorised by age, gender and *H. pylori* infection.

The overall prevalence rate of anaemia in the study children was only 15%. This prevalence rate was not significantly different between children of both age groups nor between boys and girls even after controlling for gender and age. There was a noticeable lack of association between the presence of anaemia with *H. pylori* infection and intestinal parasites. There was no difference in the mean haemoglobin concentration between *H. pylori* positive and negative children. However, it was not appropriate to examine the difference between mean haemoglobin concentration in the children with positive and negative enteric parasites.

In summary, high prevalence of *H. pylori* infection observed on study children seems depend on factors related to poor living conditions, particularly (but not exclusively) number of children, ruralism (rural-born father), the sharing a bed in childhood and type of main water supply. The results of this study indicate that low socio-economic status and/or household overcrowding may be only intermediate variables or markers for other micro-environmental factors, which may influence the mode of transmission. On the other hand, the results suggest some significant height-for-age Z-scores differences between *H. pylori* positive and negative children, as well as a significant main effect of infection on this anthropometric index. On the basis of findings from observational data, however, there is definitively not enough evidence to establish any causal relationship.

CHAPTER VI

PUBLIC HEALTH POLICY IMPLICATIONS AND RECOMMENDATIONS

H. pylori infected study children were asymptomatic carriers. Therefore, the meaning of this relatively high prevalence rate of these asymptomatic carrier children needs to be elucidated in further studies.

Although, the study's results suggest some height-for-age Z- scores differences between *H. pylori* positive and negative children, it is difficult to know whether these differences came after the infection or whether the infection was acquired recently, hence having no effect on growth. This must be true, however, in other populations living under conditions of extreme poverty, particularly suffering from simultaneous enteric parasitic infections and/or specific nutrient deficiencies together with *H. pylori* infection, which would make it of public health significance.

H. pylori infection may be one of the environmental contributory factors to linear growth retardation, rather than a primary causal factor by itself. Although positive outcomes of antimicrobial treatment on child growth have been reported, it is still unknown how much stunting on a global basis could be alleviated by efficacious chemotherapy and prophylaxis of *H. pylori* infection. The causal relationship between *H. pylori* infection and delayed growth is not clearly proven, therefore, it is premature to advocate mass treatment of children in *H. pylori*-endemic areas as a strategy to improve their growth.

Moreover, an ideal treatment for widespread infection in children has not yet been identified. Nevertheless, attention must be directed towards long-term community based treatment and prevention programmes.

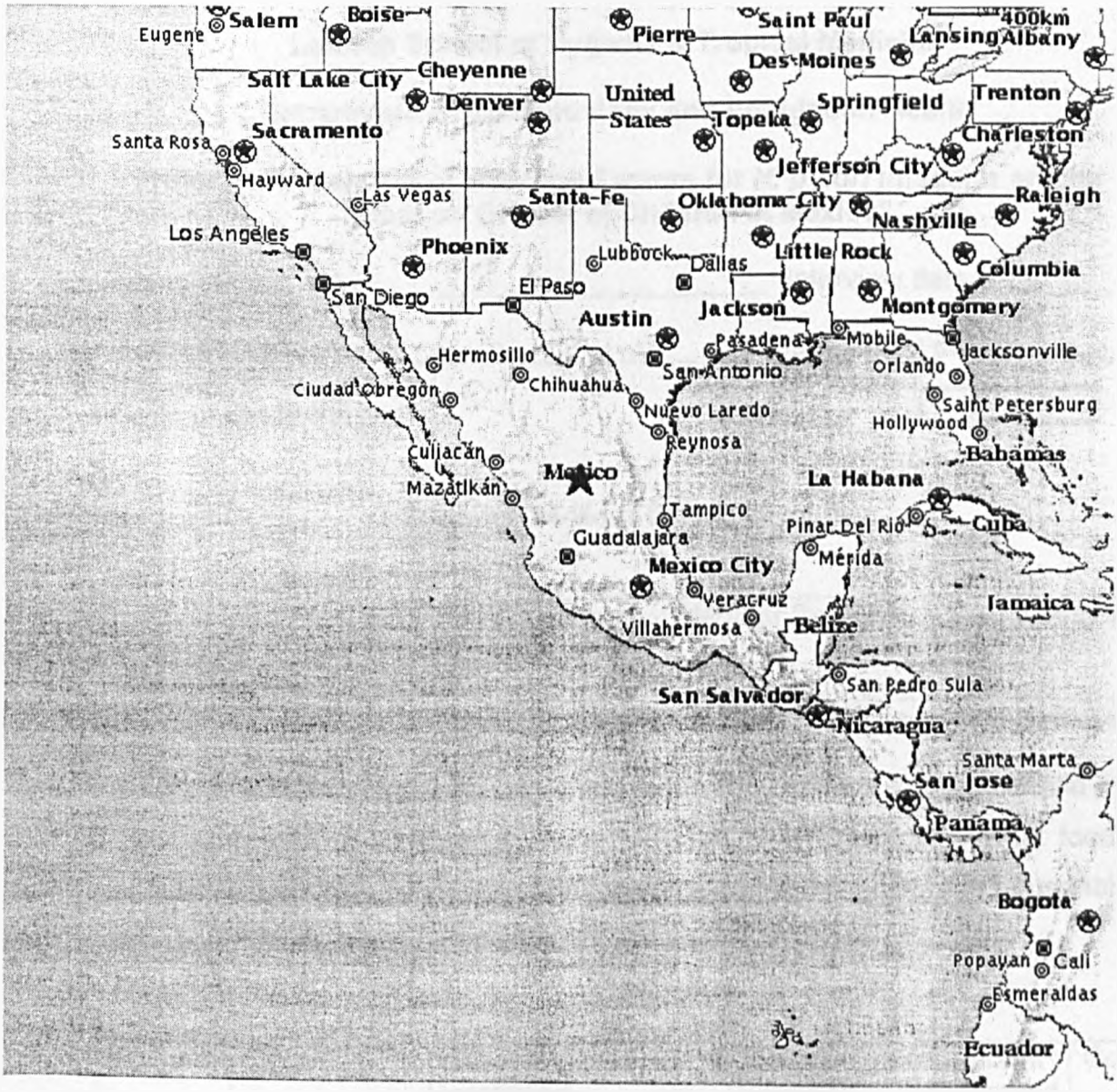
H. pylori infection may be a marker of poverty in developing countries. A related policy issue emerging from the results of this study is the question of whether, in a time of scanty economic resources in developing countries, prevention and treatment for *H. pylori* should be only targeted to particular groups on the basis of living conditions. For obvious reasons, the highest priority must be given at the level of prevention among children. The large-scale prophylactic use of antimicrobial drugs for prevention of *H. pylori*, however, is not recommended except in symptomatic children or for infected relatives of a re-infected person. Thus, understanding the ages at which children acquire infection with *H. pylori* is crucial to implement strategies for prevention.

On the other hand, relatively little is known about the extent and impact of *H. pylori* infection as a chronic health problem in children. Further studies are required to know whether *H. pylori* infection might not be malignant in certain conditions and whether eradicating the infection might be detrimental to some children.

Since large-scale accurate diagnosis of *H. pylori* is difficult in developing countries, public health strategies could attempt changes in those aspects of the community's environment or behaviour, which might be considered responsible for the high overall prevalence rate of *H. pylori* infection. Much can be done at country and regional level to improve the environmental quality in order to combat *H. pylori* infection in children.

For example, community based education programmes about good personal hygiene and handling food practices such as boiling water may be the key to prevent acquisition and transmission of *H. pylori* infection. Therefore, strategies appropriate for the local context need to be developed to target the wide spectrum of negative effects of the *H. pylori* infection.

Environmental quality is an influential direct and indirect determinant of human health. Among the major contributory factors of ill health are lack of sanitation, poor water supply, poor food safety, air pollution and poor housing. There is no doubt that household overcrowding among poor populations contributes to a less healthy environment for the children. With higher incomes, hygiene tends to improve. Thus, it is difficult to maintain high standards of hygiene for the overcrowded poor people of developing countries. In the future, the improvement of living conditions in developing countries will be the solution to prevent the spread of *H.pylori* infection.



Map of Mexico

APPENDIX B

London School of Hygiene & Tropical Medicine

Department of Epidemiology and Population Health

Project: "Prevalence of and Risk Factors for *H. pylori* Infection and its Effect on Growth of Children in Mexico"

Name of interviewer _____ Interview date_____

Name of supervisor _____ Supervision date_____

Code of Study Subject ☐☐☐

Form for 24-Hour Dietary Recall

Name of study child: _____
Father Surname Mother Surname First Name(s)

Name of mother/caretaker: _____
Father Surname Mother Surname First Name(s)

Note: I would like to ask you what you ate in the previous 24 hrs. I would be most grateful if you and your mother/caregiver could remember what foods and beverages that you consumed (including estimated amount in household measurements). In case of dishes, please describe the recipe.

No. Item	Time	Food/Beverages Description	Amount Reported	Amount Estimated	Code
1					
2					
3					
4					

APPENDIX B

No. Item	Time	Food/Beverages Description	Amount Reported	Amount Estimated	Code
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					

Name of coder: _____

Date of codification: _____

APPENDIX C

London School of Hygiene & Tropical Medicine

Department of Epidemiology and Population Health

Project: "Prevalence of and Risk Factors for *H. pylori* Infection and its Effect on Growth of Children in Mexico"

Name of interviewer _____ Interview date_____

Name of supervisor _____ Supervision date _____

Code of Study Subject ☐☐☐

Questionnaire Form on Socio-demographic Data

I.- Information of the Study Child

1.- What is his/her name? _____
Father Surname Mother Surname First Name(s)

Home address and telephone _____

Name of any relative, including its address and telephone _____

_____

2.- What is his/her date of birth? _____ Age: ☐☐
Years Months

3.- What is the sex of the child? 1 Masculine 2 Feminine ☐

4.- Where were him/her born? _____ ☐
1 Urban Community 2 Rural Community

5.- What is his/her religion? 1 Catholic 2 Protestant ☐

II.- Information of Parents

6.- Where were your born (*mother*)? _____ ☐
1 Urban Community 2 Rural Community

7.- What is your date of birth (*mother*)? _____ Age: ☐☐
Years Months

8.- Where were your husband born? _____ ☐
1 Urban Community 2 Rural Community

9.- What is your husband's date of birth? _____ Age: ☐☐
Years Months

10.- What is your current marital status? ☐

0 Cohabiting 1 Married 2 Single parent 3 Widowed 4 Divorced

III.- Information of Relatives

11.- How many other children do you have (apart of study children)? ☐

Name:	Sex (M/F)	Age

12.- Any other relatives living in your home? ☐
0 No 1 Yes

13.- How many child relatives? ☐

Name: Age: Sex (M/F) Relation to study child

14.- How many adult relatives? ☐

Name: Age: Sex (M/F) Relation to study child

IV.- Information on Occupation/Education Level of Parents

A.- Occupation of Mother

15.- What is your current occupation? ☐

0 Unemployed 1 Housewife 2 Employed

If employed, for whom do you work?

Name of company, organisation, etc.

What kind of work do you do?

What are your most important activities or duties?

16.- What is your job title?

0 No Skilled 1 Semiskilled 2 Skilled manual
3 Skilled non-manual 4 Technical 5 Professional

B.- Occupation of Father

17.- Is your husband presently employed?
0 No 1 Yes

If employed, for whom does him work?

Name of company, organisation, etc.

What kind of work does he do?

What are his most important activities or duties?

18.- What is his job title?

0 No Skilled 1 Semiskilled 2 Skilled manual
3 Skilled non-manual 4 Technical 5 Professional

C.- Educational Level of Parents

19.- What is the highest level you attended in school (not included kindergarten)?
Number of years: _____ ☐

0 No primary education 1 Primary education 2 Secondary education
3 High school 4 Technical or trade school

20.- What is the highest level your husband attended in school (not included kindergarten)?
Number of years: _____ ☐

0 No primary education 1 Primary education 2 Secondary education
3 High school 4 Technical or trade school

V.- Family Information

21.- How much was the total combined family income per month for the past 6 months? Including from jobs or any other income received $_____

Number of minimum wages ☐

APPENDIX D

London School of Hygiene & Tropical Medicine

Department of Epidemiology and Population Health

Project: "Prevalence of and Risk Factors for *H. pylori* Infection and its Effect on Growth of Children in Mexico"

Name of interviewer _____ Interview date _____

Name of supervisor _____ Supervision date _____

Code of Study Subject ☐☐☐

Questionnaire Form on Living Conditions Data

1.- How many rooms are there in your house, not including toilet? ☐

2.- Is your kitchen in a separated room? ☐
0 No **1** Yes

3.- What kind of material were used to construct the walls of your house? ☐
1 Concrete **2** Wood **3** Corrugated zinc sheet and wood
4 Corrugated cardboard sheets with a tar coating

4.- What kind of material were used to construct the roof of your house? ☐
1 Concrete **2** Corrugated zinc sheet
3 Corrugated cardboard sheets with a tar coating

5.- What kind of material were used to construct the floor of your house? ☐
1 Concrete **2** Tile **3** Dirt floor

6.- Do you have water pipe system in your house? ☐
0 No **1** Inside house **2** Outside house

7.- Is your house connected to the sewerage system? ☐
0 No **1** Yes

8.- What kind of excreta disposal in your house? ☐

0 None **1** Pit latrine **2** Toilet

9.- How many bedrooms are there in your house? _____

10.- How many persons usually sleep in each bedroom? _____

Crowding index ☐

11.- Anybody sleeping in the kitchen? ☐

0 No **1** Yes

12.- Does study child has his/her own bedroom? ☐

0 No **1** Yes

13.- Does study child has his/her own bed? ☐

0 No **1** Yes

14.- Do your other children have their own bedroom? ☐

0 No **1** Yes

15.- Do your other children have their own bed? ☐

0 No **1** Yes

16.- Do you have any pets in your house? ☐

0 No **1** Dog **2** Cat **3** Birds

Other (specify): _____

17.- Do your pets usually come into and stay inside your house? ☐

0 No **1** Yes

Which ones? _____

18.- Do your pets usually sleep inside your house? ☐

0 No **1** Yes

Which ones? _____

19.- Does study child has contact with the pets? ☐
Which ones? _____

0 No **1** Sporadically **2** Very frequently

20.- Do your other children have contact with the pets? ☐
Which ones? _____

0 No **1** Sporadically **2** Very frequently

APPENDIX E

London School of Hygiene & Tropical Medicine
Department of Epidemiology and Population Health

Project: "Prevalence of and Risk Factors for *H. pylori* Infection and its Effect on Growth of Children in Mexico"

Dear Madam/Sir

I am writing you to ask for your help in a medical research project conducted by the London School of Hygiene & Tropical Medicine. We are studying risk factors for *H. pylori* infection within families, which predispose them to infection. Our goal is to improve understanding of early acquisition of this disease and its impact on the nutritional status of children. Collection of this data will make possible for us to design public health intervention strategies. To do this we need to get information both from school children and their families.

We would appreciate your help by participating in this research. The study would involve giving information about your family through an interview, which will last about 15 minutes. Anthropometric measurements and clinical tests will be undertaken on your child, which will involve a breath analysis and 3 stool samples taken over 3 days. It is important to the success of our research that we get information from everyone we contact. All information collected will be treated confidentially, and used only in the preparation of scientific reports in which you will not be identified.

If you can help us, please sign in the end of the letter. I look forward to hearing from you soon.

Thank you for your help

Yes ☐ Accept to participe: _____

No ☐

APPENDIX F

London School of Hygiene & Tropical Medicine

Department of Epidemiology and Population Health

Project: "Prevalence of and Risk Factors for *H. pylori* infection and its Effect on Growth of Children in Mexico"

Name of the study child: _____

REPORT WITH THE STUDY RESULTS

I.- Anthropometry A.- Height-for-age B.- Weight-for-age C.- Weight-for-height	 103.25 % 109.18 % 101.38 %	**Diagnostic:** Normal
II.- Haemoglobin concentration	11.2 g/dl	**Diagnostic:** Anaemia
III.- Faecal Examination A.- Protozoans: *Endolimax nana* *Entamoeba coli* *Giardia lamblia* *Iodamoeba bütschlii* *Entamoeba histolytica* B.- Helminths: *Hymenolepis nana* *Ascaris lumbricoides* *Hymenolepis diminuta* *Trichuris trichiura*		**Result:** Negative Negative Negative Negative Negative Negative Negative Negative Negative
IV.- *Helicobacter pylori* status		**Result:** Positive

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